Author:
Pegler, Katherine Ruth

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Morphology and behaviour of parasitic Psoroptes mites (Acari : Psoroptidae)

Morphology and behaviour of parasitic *Psoroptes* mites (Acari: Psoroptidae)

by

Katherine Ruth Pegler

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ABSTRACT

The astigmatid mite, *Psoroptes ovis* (Hering) (Acari: Psoroptidae) is an obligate, non-burrowing ectoparasite of vertebrates. It infests a wide range of hosts but is of greatest clinical and economic importance in sheep. Control of the mites currently relies on organophosphate and pyrethroid dips and injectable macrocyclic lactones. However, concerns over the effects these compounds on human health, the environment and the development of resistance is stimulating renewed interest in the development of novel control technologies. One limiting factor affecting such developments is that *Psoroptes* mites can currently only be reared on a vertebrate host. Hence trials of new control methods require the use of live animals to supply mites which raises animal welfare issues and is expensive. The aim of the work described in this thesis was to examine aspects of the morphology and behaviour of *Psoroptes* mites ultimately with a view to contributing to the development of *in vitro* rearing of mites.

Morphological comparison of adult mites was carried out to determine whether it was reasonable to extrapolate from the behaviour of *Psoroptes* mites obtained from one host to *Psoroptes* mites from another host (Chapter 3). Measurements of morphological characters from adult male and female mites showed that there were some broad but significant differences in shape and size between mites from different hosts and that the length of the outer opisthosomal setae was the most important distinguishing character in both sexes. However, a large degree of variation in morphology was observed and size did not appear to be host, geographic or body site specific. It is concluded that mites infesting different hosts cannot be reliably classified as different species on the basis of morphology.

The first stage in considering *in vitro* rearing was to examine the responses of the mites to light, temperature and gravity (Chapter 4). When placed on a horizontal surface of uniform temperature and illumination, temperature had a significant linear relationship with linear velocity and angular velocity. On a vertical surface movement of the mites was strongly directed towards areas of high temperature but away from high light intensity. In the absence of any unidirectional light gradient, mites moved upwards. The movement of the mites in response to the temperature gradient was strongly displaced up or down by the presence of unidirectional illumination from above or below, respectively. These results allow the behaviour of mites to be controlled *in vitro* using combinations of light and temperature gradients.

Finally to attempt to achieve *in vitro* survival, feeding and reproduction, the effect of various environmental conditions and diets were considered (Chapter 5). When maintained at a constant humidity, temperature had a significant effect on the LT_{50} and maximum survival of adult female mites, the rate of oviposition, the maximum period of oviposition, the time taken for eggs to hatch and larval survival time. Adult female LT_{50} and maximum survival, rate of oviposition and maximum period of ovipostion were all maximised when maintained at 30°C. Both the time required for eggs to hatch and larval survival time decreased with increasing temperature. When maintained at constant temperature and humidity, the diet with which mites were provided had a significant effect on the LT_{50} and maximum survival of adult female mites, the rate of oviposition, the time taken for eggs to hatch and larval survival time. However, no single diet provided consistent significant improvements in mite survival and development. Photoperiod had a significant effect on adult female LT_{50} and maximum survival, the rate of oviposition, the time required for eggs to hatch and larval survival time. Survival and development of mites was maximal under complete darkness.

It is concluded that the behaviour of mites *in vitro* can be manipulated and longevity increased when mites are provided with appropriate environmental conditions and food sources. Mites will feed, oviposit and eggs will hatch *in vitro*. However, the one major limiting step appears to be the transition from larvae to protonymph; larvae die rapidly and do not moult *in vitro*. Clearly this life-cycle stage now needs to be the focus of greater attention to allow the problem of *in vitro* rearing to be overcome.

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AUTHOR'S DECLARATION

I declare that the work in this dissertation was carried out in accordance with the Regulations of the University of Bristol. The work is original except where indicated by special reference in the text and no part of the dissertation has been submitted for any other degree.

Any views expressed in the dissertation are those of the author and in no way represent those of the University of Bristol.

The dissertation has not been presented to any other University for examination either in the United Kingdom or overseas.

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APPENDICES: PUBLISHED PAPERS

Pegler, K.R. and Wall, R. (2004). Tactic responses of the parasitic mite, *Psoroptes ovis*, to light and temperature. *Experimental and Applied Acarology* 33: 69-79.

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CHAPTER 1

INTRODUCTION

1.1 Systematics

The astigmatid mite, *Psoroptes ovis* (Hering) (Acari: Psoroptidae), is an obligate, non-burrowing ectoparasite of vertebrates. It is distinguished by the presence of a terminal sucker on a relatively long, three segmented pre-tarsus (Hirst, 1922). It infests a wide range of hosts, including cattle, goats, rabbits and horses but is most well known and of greatest clinical and economic importance when it is found on sheep. Despite being one of the oldest known diseases of sheep, the causative agent was only discovered in 1809 by G.H. Waltz, a veterinary surgeon in Germany (cited in Downing, 1936a). The mite was first described and named *Sarcoptes ovis* by Hering in 1838, but it was later assigned to the genus *Psoroptes* by Gervais in 1841.

Following the initial description from sheep, as many as nine species of *Psoroptes* mite were proposed, each distinguished from the others mainly by the different mammalian hosts they infest. However, because of the high degree of morphological similarity between the proposed *Psoroptes* species, some early authors believed that there was in fact just a single species, synonomised initially as *Dermatokoptes communis* by Fürstenberg in 1861.

In 1877, Megnin advocated a return to the genus *Psoroptes* and subsequent authors referred to populations of mites as *Psoroptes communis* with varietal names such as *ovis* or *bovis* appended, depending on the host species from which they were obtained (Raillet, 1893; Stockman & Berry, 1913; Shilston, 1915). Hirst (1922) found little morphological difference between mite populations from different

species of host, providing support for the description of all mites infesting sheep, goats, cattle, horses and rabbits as *Psoroptes communis.* However, some morphological differences were found in *Psoroptes natalensis* which has opisthosomal setae that are blade-like and flattened at the distal end and was first described from specimens found on cattle.

A detailed morphological study was carried out by Sweatman in 1958 who proposed that there were five species of *Psoroptes,* and produced a key, distinguishing between the putative species using the length of the outer opisthosomal seta of adult male mites.

1.2 Pathology and disease

Initial infestation of sheep with *P. ovis* can be difficult to detect. The first lesions may appear within 2 days (Kirkwood, 1980) and take the form of small yellow vesicles of serous exudate surrounded by areas of inflamed skin, that can be seen only when the fleece is parted (Tarry, 1974; Kirkwood, 1980; Bates, 1997)(Fig. 1.1a). The vesicles expand and rupture and body heat from the host causes the centre of the lesion to dry out leading to the characteristic scab (Bates, 1997). The hardened scab appears to be an unsuitable habitat for the mites as they are seen to congregate at the edge of the lesion (Babcock and Black, 1933; Kirkwood, 1986; Bates, 1997) causing the lesion to increase further in size. The lesions cause severe irritation to the sheep causing them to become restless and to bite and rub the infested area (Babcock and Black, 1933; Corke and Broom, 1999; Berriatua *et al.,* 2001). The fleece appears soiled and ragged and much of it may be lost (Fig. 1.1b) both by the lifting away of old lesions and by the continual rubbing and biting by the host (Tarry, 1974; Kirkwood, 1980; van den Broek and Huntley, 2003). The disease may result in the death of the host within 8-12 weeks (Berriatua *et al.,* 1999, 2001). As well as

severe welfare issues, the disease can result in great financial loss, largely as a result of weight loss of the animal. Sargison *et al.* (1995) found a 10% reduction in birth weight of lambs born to ewes with severe sheep scab compared to those with mild cases of the disease. Kirkwood (1980) found a weight loss of 30% and a loss of 1000 cm^2 (0.2kg) of wool in nine-month-old sheep artificially infested with 25 mites over a 14 week infestation period compared to healthy animals. Kirkwood (1980) calculated that a 30% weight loss would equate to a severe financial loss, costing £1000 in a flock of 100 infested animals. It was later calculated that over a 30 year period without government control, sheep scab could cost the UK up to £600 million (Kirkwood, 1986).

In domestic rabbits infestation with *Psoroptes* mites is most commonly seen as ear canker, where the infection is confined to the ear canal and the pinnae (Fig. 1.2) and extra-auricular mange, where the infestation may spread over the body of the animal (Bates, 1999). Infestation begins deep in the external auditory canal and may remain sub-clinical for long periods of time before becoming apparent. *Psoroptes* mites can infest both domestic and laboratory animals (Strong and Halliday, 1992; Bates, 1999). Initial symptoms take the form of small, moist lesions where the corneum appears eroded, exposing the granular layer of the skin (Meleney, 1967). These small lesions develop to form crusts and scabs which can spread to cover the whole internal surface of the pinna (Meleney, 1967; Bates, 1999; Perrucci *et al.*, 2001). In the case of extra-auricular mange, which only appears to occur in a minority of cases, with only 5% of infested rabbits examined at the Veterinary Laboratories Agency having lesions outside of the ears, lesions may spread to cover the base of the ears, cheeks, dewlap, face and between the digits of the hind feet (Bates, 1999).

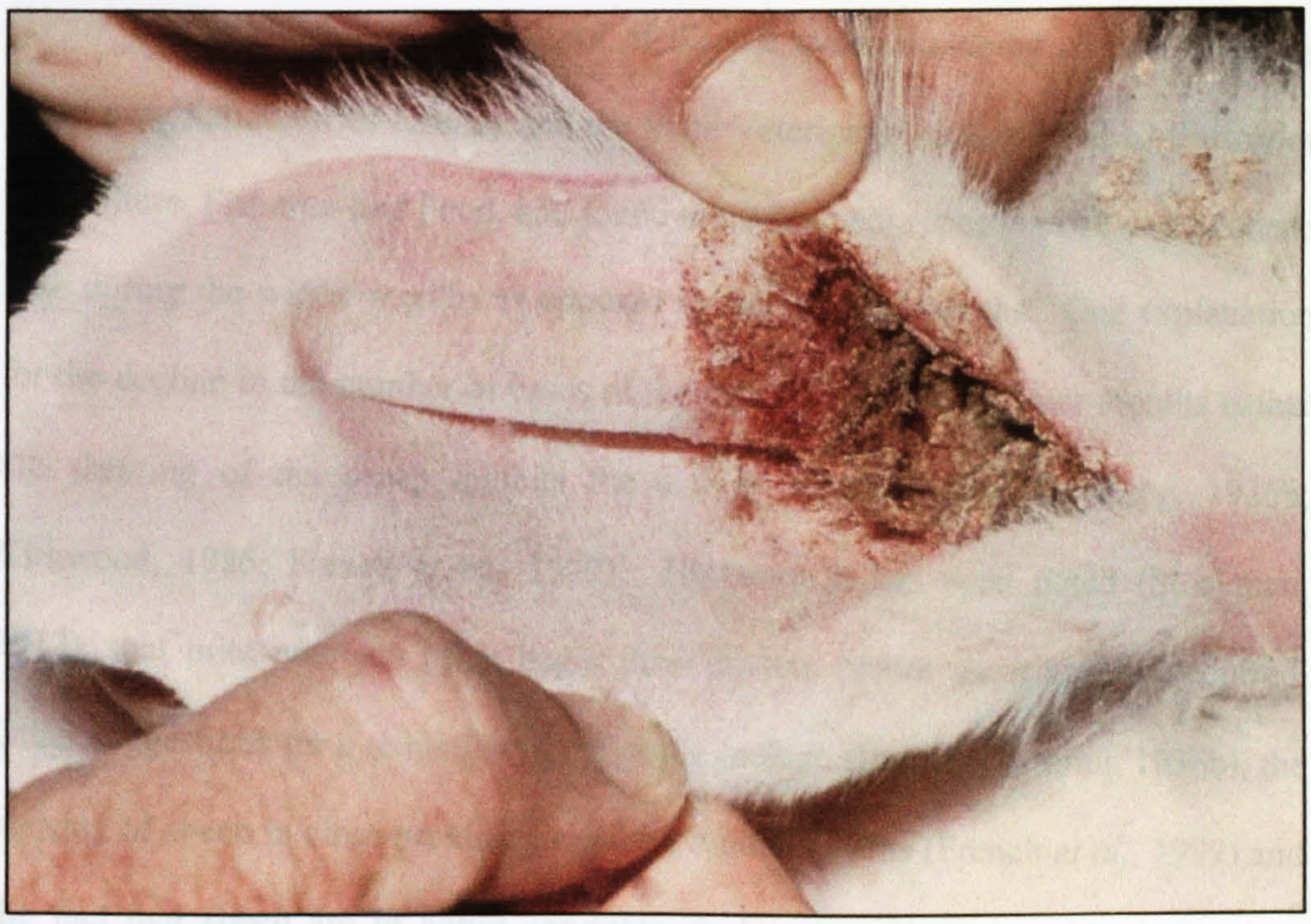
The means by which the mite causes the clinical disease is still not entirely known or understood. Early authors believed that the clinical symptoms were the direct result of feeding of the mites and mechanical damage caused by the chelicerae (Shilston, 1915; Downing 1936a). It was thought that the mites pierced the skin causing irritation and the formation of vesicles and pustules which then ruptured causing crusts and the shedding of wool. However, a 96% reduction in mite population size, using a subcutaneous injection of ivermectin, failed to slow down lesion growth, leading to the conclusion that clinical disease was not a result of mechanical feeding damage, but the result of an allergic response thought to be caused by antigenic material found in the mites faeces (Bates and Groves, 1991). Antigens have been found in faecal material from other species of mite, including the house dust mite (Tovey *et al.*, 1981) and antigens found in *Psoroptes* have been shown to be homologous to those of the house dust mite group 1 allergens (Lee *et al.* 2002).

Figure 1.1 Small yellow vesicles of serous exudates surrounded by areas of inflamed skin seen in initial stages of infestation with *Psoroptes* mites (a) and a severe case of scab in a sheep showing extensive wool loss (b). Photographs courtesy of E. Berriatua.

(a)

(b)

Figure 1.2 Lesion resulting from auricular infestation of a rabbit with *Psoroptes*. Origin of photograph unknown.



Sheep scab has long been regarded as a winter disease, as although infestation may occur throughout the year, incidence is usually higher during the winter months (Downing, 1936b; Kirkwood, 1986; French *et al.*, 1999). Fourie *et al.* (2002) found an increased rate of lesion growth on groups of five or ten Merino and Dorper sheep when artificially infested in the winter months than in the summer. French *et al.* (1999) looked at the seasonal pattern of disease outbreaks using records from the Veterinary Laboratories Agency and the State Veterinary Service for the Ministry of Agriculture, Fisheries and Food, and found more frequent reporting of cases of sheep scab during the winter months as opposed to the summer months. One explanation for the decline in the number of cases of the disease over the summer months is that the shearing of the sheep inhibits the activity of the mites (Downing, 1936b; Kirkwood, 1986; French *et al.*, 1999). However, it has been noted (Stockman, 1912), that mite numbers often begin their decline before shearing has occurred. Other suggestions for a reduction in incidence include climate (Downing 1936b), the housing of sheep in close proximity over the winter months (French *et al.*, 1999) and the fact that sheep are in better condition during the summer, making them less susceptible to infestation (Kirkwood, 1986).

Downing (1936a,b) suggested that during the summer months, mites enter a "latent" phase, remaining dormant in "cryptic" sites, including the infra-orbital fossae, the external auditory canal or under the secretions and crusts of the skin glands (Downing, 1936a,b). During this latent phase, the animal appears to recover and the wool regrows. Observations of infested sheep show that it is possible for mites to be present on an animal, but for the animal to show no symptoms that could be easily detected, for up to six months (Babcock and Black, 1933).

1.3 Life-history

Psoroptes mites undergo their entire life cycle on the host (Kirkwood, 1986). There are five life cycle stages: egg, larva, protonymph, tritonymph and adult (Sweatman, 1958; Sanders *et al.*, 2000). The time taken for a complete egg to adult life cycle has been estimated to be 10.7 days (Downing, 1936b; Wall *et al.*, 1999), 11 days (Shilston, 1915), 16 days (Stockman, 1910) and 14-19 days (Sweatman, 1958).

The eggs are relatively large compared to the size of the adult female (Fig. 1.3a). They have been recorded to take 2 – 3 days to hatch (Shilston, 1915; Downing, 1936b; Wall *et al.*, 1999). Larvae show no sexual dimorphism and range in length from 0.250 – 0.322 mm (Sweatman, 1958; Sanders *et al.*, 2000; Fig. 2.5). The larval stage lasts for, on average, 2.2 days (Downing, 1936b; Wall *et al.*, 1999) before moulting into the protonymph stage (Figs. 2.3a and 2.4a). Sexual dimorphism is observed in the nymphal stages with the male protonymph being slightly larger (0.313 – 0.353 mm in length) than the female protonymph (0.309-0.346 mm in length) (Sweatman, 1958; Sanders *et al.*, 2000). Tritonymphs (Figs. 2.3b and 2.4b) are significantly larger than the protonymphs with the male nymph (0.414 – 0.455 mm in length) again being slightly larger than the female (0.402 – 0.436 mm in length) (Sweatman, 1958; Sanders *et al.*, 2000). The protonymph and tritonymph stages last for 2.3 and 2.2 days, respectively (Downing, 1936b; Wall *et al.*, 1999). Adult females (0.536 – 0.568 mm in length; Fig. 2.1) are significantly larger than adult males (0.396 – 0.472 mm in length; Figs. 1.3b and 2.2) with the adult female being morphologically more similar to the immature stages than the adult male (Sweatman, 1958; Sanders *et al.*, 2000). Adult males will attach to female nymphal stages (Sweatman, 1958). This pairing is most commonly seen between males and female tritonymphs although attachment to female protonymphs is also observed (Guillot and Wright, 1983). It is believed, however, that copulation does not occur

until the female has moulted to the adult stage (Guillot and Wright, 1983). The formation of these attachment pairs is thought to increase reproductive fitness of the male mites, with the probability of a successful mating increasing for males attached to a female tritonymph (Sweatman, 1958; Guillot and Wright, 1983). Once a female has reached the adult stage, it takes on average 1.3 days until the first egg is laid. After this pre-oviposition period, a female will then produce an average of 2.9 eggs per day. However, the number of eggs produced by a particular female has been shown to be negatively related to female age, with the number of eggs produced by a female in a 24 h period declining as the mite gets older (Downing, 1936b; Wall *et al.*, 1999).

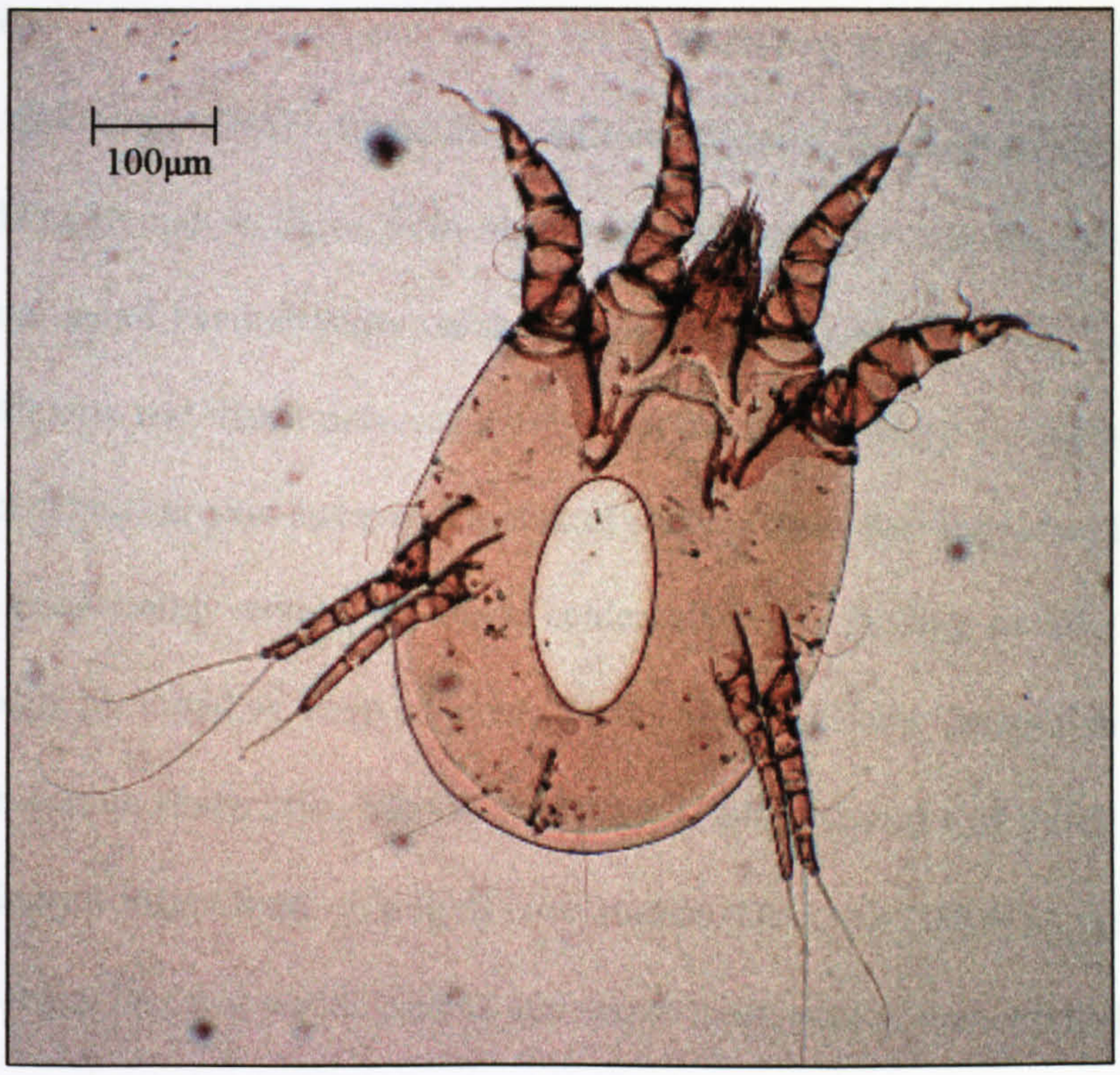
1.4 Biology

It was suggested that *Psoroptes* infesting sheep directly pierced the skin surface (Shilston, 1915) and later authors supported this view though they did not however, provide evidence that the mites could indeed pierce the epidermis (Sweatman, 1958; Tarry, 1974). Blake *et al.* (1978) carried out the first detailed study of the mouthparts of *Psoroptes*. It was suggested that the mites lack salivary glands and as a result it was proposed that the mites do not penetrate the host skin but obtain liquid food by abrading caused by toothed chelicerae. A later study carried out by Rafferty and Gray (1987) used scanning electron microscopy to show that the mouthparts of *Psoroptes* mites from rabbits and sheep were identical in morphology and supported Blake *et al.* (1978) in that the mouthparts were adapted for surface feeding rather than piercing. Examination of frozen skin sections using light microscopy and freeze fractured material using scanning electron microscopy failed to reveal embedded mite mouthparts or tissue damage, thus suggesting that mites do not pierce any deeper than the outermost loose stratum corneum (Sinclair and

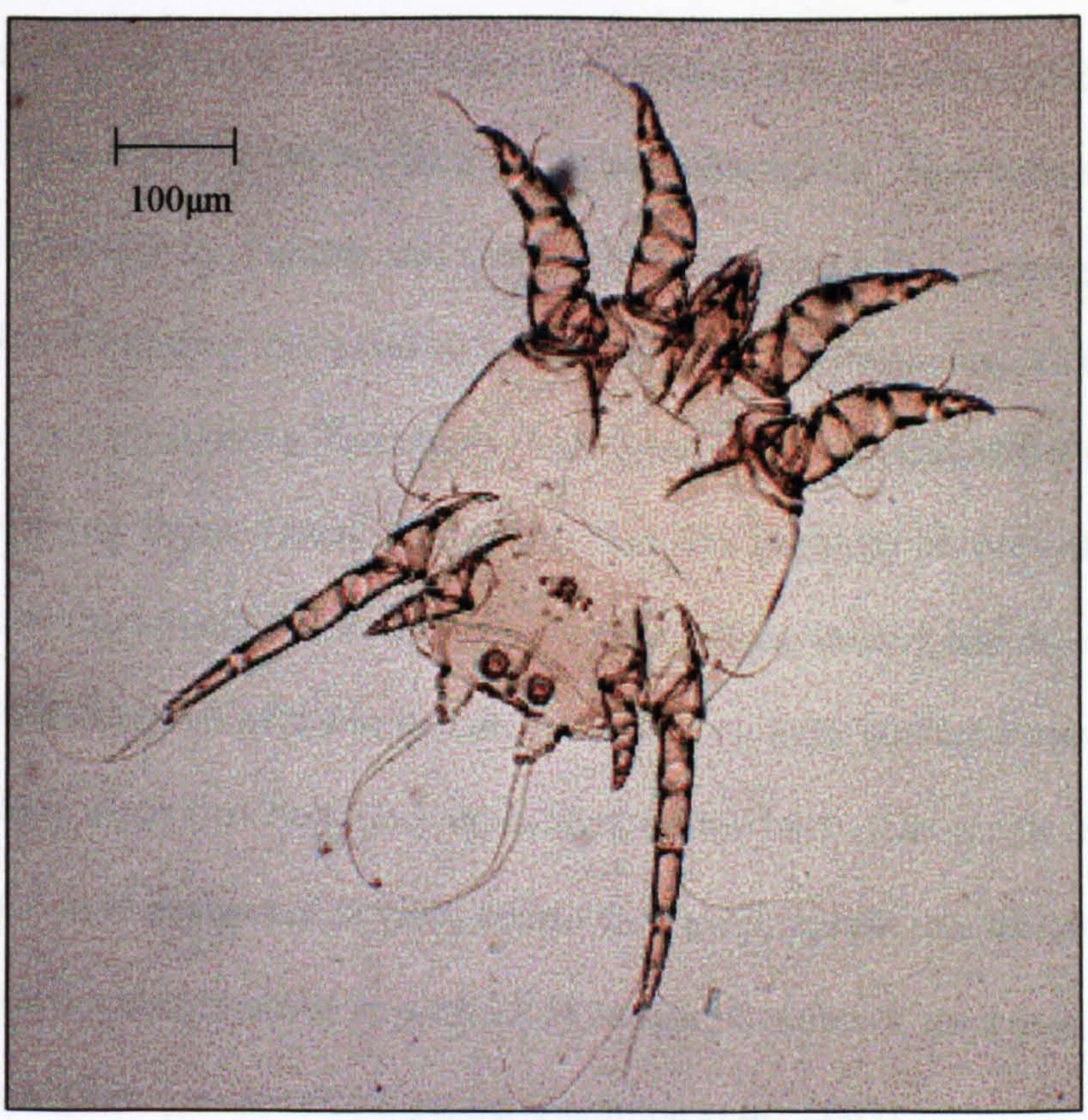
Kirkwood, 1983). Although it has previously been suggested that *Psoroptes* mites do not possess salivary glands (Blake *et al.*, 1978), Rafferty and Gray (1987) noted two distinct pores, located dorsally to the labrum that were proposed to be the openings of ducts to the salivary glands. Following a detailed study of *Psoroptes* mouthparts using scanning and transmission electron microscopy, Mapstone *et al.*, (2002) also suggested that the mites may move saliva onto the host skin whilst simultaneously ingesting food. It was proposed that a two-way flow of liquid occurs with saliva flowing down a central salivary canal between the two chelicerae with liquid food moving, initially by capillary action, along the groves of the pseudorutellae from which it can be sucked up a food canal in the pre-oral trough by the pump-like action of the pharynx.

Figure 1.3. Photographs of cleared adult female (a) and adult male (b) *Psoroptes* mites viewed under a phase contrast light microscope.

(a)



(b)



It is now largely accepted that *Psoroptes* mites feed superficially on the lipid emulsion of lymph, skin cells, secretions and bacteria on the surface of the host skin (Blake *et al.*, 1978; Raferty and Gray, 1987; Mapstone *et al.*, 2002). However, Rafferty and Gray (1987) found haemoglobin content in *Psoroptes* mites from rabbits although not in those from sheep. They proposed that the haemoglobin comes from small haemorrhages on the skin surface as a result of abrasion by the mite mouthparts and that it only occurs in mites infesting rabbits due to the relative thinness of rabbit ear skin compared to that of sheep. They also suggested that mites on sheep feed mainly within the outer epidermal layer, feeding mainly on lipid, which would be found in large amounts in the fleece. Using lipophilic stains to colour the lipid in sheep skin, Sinclair and Filan (1989) showed that mites infesting sheep do indeed ingest lipid. Using *in vitro* studies, DeLoach (1984) suggested that *Psoroptes* mites ingested water, plasma and serum more readily than whole blood, as more mites were observed to feed on these diets, however, no significant differences were found. DeLoach and Wright (1981) used radioactively labelled erythrocytes to show that mites infesting rabbits did ingest whole blood cells. However, scanning electron microscopy by Rafferty and Gray (1987) could only find haemoglobin in mite samples, suggesting that mites only ingest erythrocyte fragments. Using SDS PAGE and Western Blotting Techniques, Mathieson (1995) showed that *Psoroptes* mites from sheep ingest serum components that are likely to be present in the secretions produced in clinical sheep scab. A model of the feeding and digestion of *Psoroptes* mites supports evidence that the mites feed on loose stratum corneum and lipid secretions when first becoming established on the host. The model suggests that epidermal lipid is ingested as mites embed their gnathosomes into the epidermis and once skin breakages occur as a result of host inflammatory responses, serum exudates and erythrocytes are also ingested (Hamilton *et al.*, 2003).

In both cattle and sheep, it has been suggested that a range of populations of *Psoroptes* mites exist, with varying degrees of pathogenicity (Roberts and Meleney, 1971). These strains may cause an acute or chronic disease, with the chronic strains being more readily affected by the host's immune system. The acute strains are able to spread rapidly through populations of livestock. Mathieson and Lehane (1996) suggested that this variation in pathogenicity may be attributable to the intestinal flora of the mite. Bacteria discovered in the gut of *P. ovis* is not naturally found on the sheep suggesting that it is not a result of dietary intake but must be associated with the mite in some way.

Although the whole lifecycle of *Psoroptes ovis* can occur on a single host, transmission occurs rapidly throughout a flock of naïve hosts. A flock of nine sheep were all found to show lesions after contact with a single infected individual within 8-14 weeks (Meintjies *et al.* 2002a). Transmission may occur as a result of contact with an infested animal or an infested environment, such as sheep housing or scratching posts (Wilson *et al.*, 1977). When groups of between 6 and 20 healthy sheep were housed with a single mite infested animal it was found that the probability of transmission peaks at the time when the population of adult mites on the original infested animal reaches its peak abundance. This peak in mite abundance occurred 9-11 weeks following infestation (Berriatua *et al.*, 1999).

Wilson *et al.* (1977) looked at the transmission of mites that had become detached from the host. Transmission was examined by leaving housing, which had been occupied by 10-20 infected sheep, vacant for varying lengths of time and then introducing groups of naïve individuals. It was found that transmission did not occur to clean individuals when housing had been left vacant for more than 72 hours.

In a similar experiment, Stockman (1912) placed heavily infected sheep in a pen large enough to house one or two sheep comfortably. The precise number of

infested animals used or the amount of time they were housed for are not stated. Infested animals were removed and the pens left vacant for 2, 7, 8 or 14 days before introducing healthy individuals. It was found that the pen could be left vacant for up to 8 days and a new infection could still be initiated. However, infection in naïve hosts was not reliable as, of the seven cases where the pen was left vacant for 7 days, only once was there a successful infection and only once was the pen left vacant for 8 days. Stockman does not state when these trials were carried out so perhaps seasonal changes in temperature were able to alter the length of infectivity of the mites.

When considering both of these studies it appears that transmission may occur naturally when the mite is off the host for no more than 8 days, less than half of the time that the mite has been seen to survive outdoors off the host. Wilson *et al.* (1977) compared natural and artificial transmission. In the latter case mites were placed directly on the skin of the sheep. It was found that mites that had been off-host for up to 12 days could initiate an infection. Similarly, O'Brien *et al.* (1994a) found that mites could retain infectivity when removed from the host for up to 15 days. This suggests that although mites are able to initiate infection when placed directly on the host after off-host storage for up to 15 days, they are unable to reach a new host and successfully establish a natural infection when they have been off-host for more than 8 days.

Field and laboratory studies have shown that the survival of *P. ovis* is enhanced at lower temperatures (Shilston, 1915; Wilson *et al.*, 1977; Liebisch *et al.*, 1985; Smith *et al.*, 1999) suggesting that field survival of the mites may be greater in colder weather and therefore increasing the chance of transmission. It has also been observed in the laboratory that it is the adult females that have the greatest longevity (Liebisch *et al.*, 1985; Smith *et al.*, 1999) and it has been shown experimentally that

eggs are not able to retain vitality when separated from the host for any more than eight days (Stockman, 1912; Shilston, 1915; O'Brien *et al.*, 1994a). This suggests that it is the adult females that are the persisting phase and responsible for the initiation of new infections.

1.5 Prevalence and distribution

Sheep scab has been known in Britain for several hundred years. The first prevalence data are from 1807 when there were 2,573 outbreaks; the number rose to 3,536 outbreaks in 1895 (Kirkwood, 1986). In 1905 it became compulsory to dip all scabby sheep in government approved dips, thus leading to a reduction in outbreaks. There were just 226 cases in 1914 (Kirkwood, 1986). Sheep scab was finally thought to have been eradicated from Britain in 1952, but new cases of the disease were found in Lancashire in 1973. It is believed that the reintroduction was a result of the importation of infested sheep, possibly from Ireland (Loxam, 1974). In early 1973, 27 outbreaks were reported with a further 21 cases observed during the winter of 1973-1974 (Tarry, 1974). Compulsory dipping of sheep was reintroduced in 1976, as the disease spread throughout Britain (Kirkwood, 1986). This compulsory autumn dipping caused the numbers of outbreaks to decline (Fig. 1.4). However, a switch to summer dipping in 1983 resulted in an increase in the number of cases reported (French *et al.*, 1999). The introduction of twice-yearly dipping in 1984 caused another decline in the number of cases of sheep scab but, in 1989, the compulsory summer dip was abandoned causing the number of outbreaks to increase once more (Fig 1.4). Overall, between 1973 and 1992, a total of 1,480 flock outbreaks of sheep scab were recorded, with almost all counties in England, Wales and Scotland reporting outbreaks (French *et al.*, 1999). In 1992, sheep scab was de-

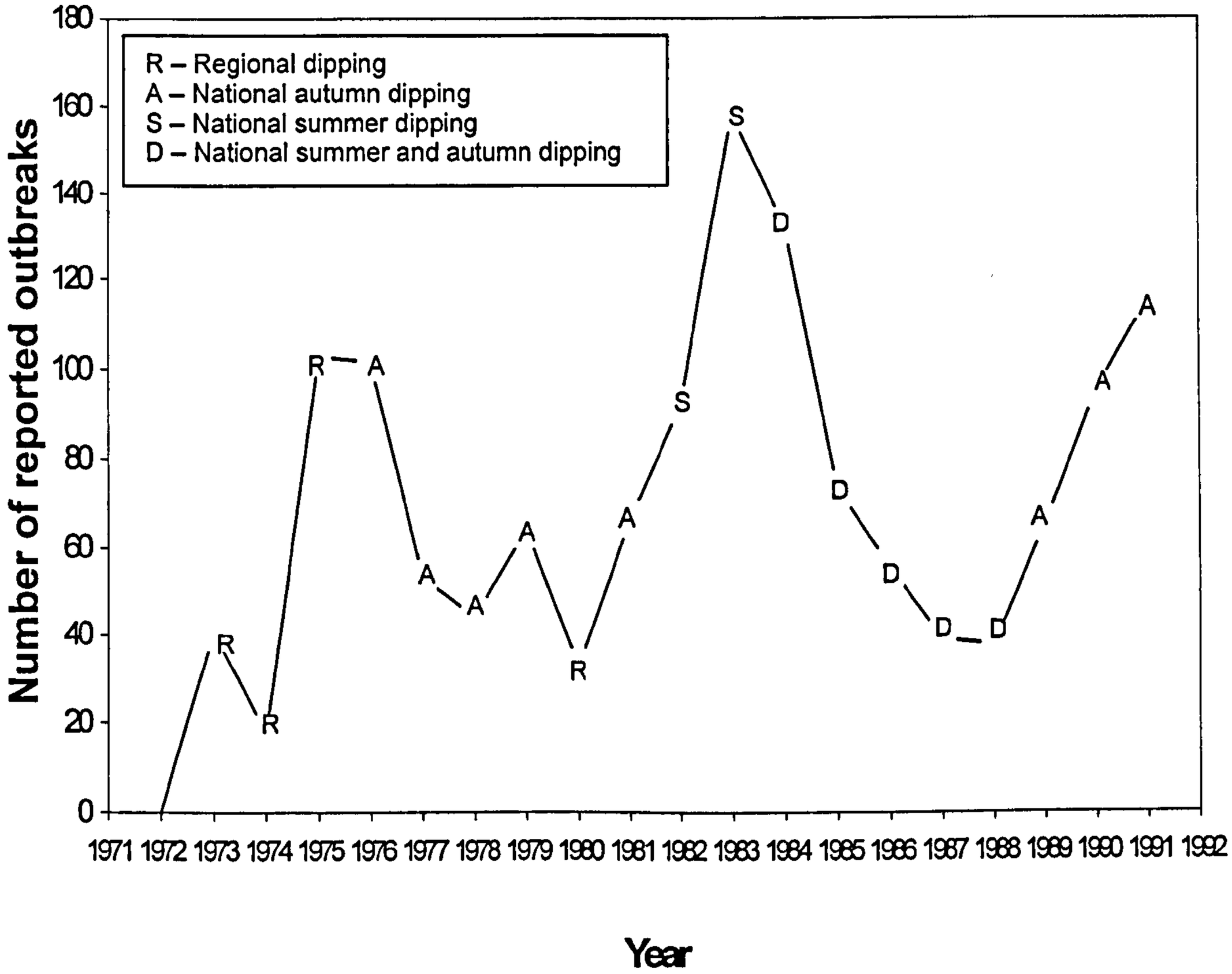
regulated and the sheep industry left to control the disease itself. As a result of this, accurate record collection ceased.

A postal questionnaire survey in 1999 received 1,100 completed questionnaires from sheep farmers who were members of the National Sheep Association. Using the number of claims for Sheep Annual Premium in 1999 as the number of farms in Britain, it was estimated that 5000 farms are likely to have been affected by sheep scab in a 12-month period from October 1998 to September 1999 (Corke and Broom, 2000). A similar postal survey in 2004 received 1,067 replies from sheep farmers in Scotland, England and Wales. Overall, 9% of farms that took part in the survey had at least one case of scab between March 2003 and February 2004. This gives an estimate of the number of outbreaks throughout Britain to be nearing 7000 in the year (Bisdorff *et al.*, 2005).

1.6 Control

Sheep scab has been successfully eradicated in a number of countries including Australia in 1884 (Seddon, 1964), Norway in 1894, New Zealand in 1885, Canada in 1927, Denmark in 1929, Sweden in 1934 (Kirkwood, 1986) and is thought to have been eradicated from North America in 1973 (Graham and Hourrigan, 1977). Eradication in Australia was achieved by the euthanasia and burning of all infested sheep and their contacts, along with the burning of fencing and hurdles used by infested animals (Seddon, 1964). Eradication of the disease in sheep in North America coincided with a decline in the number of sheep suggesting that control efforts were assisted by a reduction in the number of sheep that needed treatment (Meleney and Christy, 1978).

Figure 1.4 The number of reported outbreaks of sheep scab per year from January 1973 to June 1992 in Great Britain as recorded by the Veterinary Laboratories Agency and the State Veterinary Service for the Ministry of Agriculture, Fisheries and Food. (Redrawn from French *et al.*, 1999).



Early control methods in Britain included the washing of sheep in olive oil dregs, water in which lupins had been soaked or washes containing lime, mercury, sulphur or arsenic (Kirkwood, 1985, 1986; O'Brien, 1999). The first commercial dip, developed by William Cooper in 1843, used a wettable powder containing arsenic and sulphur. When compulsory dipping was introduced in 1906, many of the compounds used, although effective in killing the mites, caused staining and damage to fleeces and could result in weight loss in the sheep, and were thus uneconomic. Furthermore, such compounds did not kill the mite eggs and did not persist in the fleece long enough to kill newly hatched larvae, therefore double dipping, with dips occurring within 10-14 days of each other, was required and was introduced in 1914 (Kirkwood, 1985).

Downing (1947) was the first to test organochlorines, including DDT and lindane (gamma-HCH), as potential dips and found that they could give up to three months residual activity, thus providing a major advance in the insecticidal control of the disease. Lindane was approved as a dip in 1948 and its use is believed to have made a major contribution to the eradication of the disease within 4 years of its introduction.

Following reintroduction of the disease in 1973 (Loxam, 1974), and the Government controlled eradication campaign of compulsory dipping of all sheep, in 1981, the organophosphate diazinon was approved as a scab dip along with propetamphos, (Kirkwood and Quick, 1981, 1982). Both became used increasingly after 1985, when organochlorines were withdrawn due to concerns over meat residues and environmental contamination (O'Brien, 1999). The pyrethroids have been used both as dips and pour-on formulations against a range of ectoparasites since the early 1980s (O'Brien, 1999). The first pyrethroid to be licensed for use in the UK against sheep scab was flumethrin which was introduced as a dip in 1987

(Kirkwood and Bates, 1987). However, flumethrin is no longer licensed in the UK, France or the Republic of Ireland (Bates, 2004), leaving high cis-cypermethrin as the only pyrethroid currently approved for use in the UK.

Since 1992, there has been growing pressure to limit, if not ban altogether, the use of conventional neurotoxic dips. This pressure has come from farming groups concerned with the possible effects, particularly of organophosphates, on human health and also from lobbies concerned with environmental contamination by agrochemical pesticide residuals. Stephens *et al.* (1995) examined the neuropsychological performance of 146 sheep farmers who had experience long-term low level exposure to organophosphates. They found that exposed farmers performed significantly worse in tests of attention and speed of information processing compared to an unexposed control group. A similar study looking at 612 sheep farmers, found a weak association between organophosphate exposure and neurological symptoms. However, much greater neurological effects were observed in farmers that had handled the organophosphate concentrate, suggesting that acute organophosphate poisoning is more likely to result in neuropsychological abnormalities than long-term low-level exposure (Pilkington *et al.*, 2001). A similar result was found using a telephone survey of 367 individuals who believed they had been made ill as a result of organophosphate exposure. It was concluded that most reported illness was among those who had experienced high-level exposure, as opposed to those who have had lifetime cumulative exposures (Fletcher and MacLehose, 2005).

In addition to fears over organophosphate use, alternative methods for scab control are also needed to overcome problems of the development of resistance of *Psoroptes* to the existing pesticides, with confirmed cases of resistance to synthetic

pyrethroid and organophosphate dips (Synge *et al.*, 1995; Clark *et al.*, 1996; Coles and Stafford, 1999).

The macrocyclic lactones were first developed in the mid 1970s, with the compound ivermectin being used as an anthelmintic. Since then, they have been the focus of increasing interest for use in the control of sheep scab. Ivermectin is effective against sheep scab following two subcutaneous injections of 200μg/kg, seven days apart (Soll *et al.*, 1992). Two further compounds have also been found to be effective with a single subcutaneous injection; moxidectin at 200μg/kg (O'Brien *et al.*, 1994b) and doramectin at 300μg/kg (Bates *et al.*, 1995). The main advantages of a systemic acaricide such as ivermectin are that injection is a fast and safe alternative to dipping, causing less stress to the sheep and producing less chemical waste (Bates, 1993). However, due to the relatively short residual activity, particularly of ivermectin, great care must be taken to administer the correct dose to each animal, failure to treat a single animal may result in reinfestation and prove costly (O'Brien, 1999). Also, macrocyclic lactones are relatively slow acting, have long withdrawal periods for meat and milk and have an effect on a narrower range of ectoparasites than dips (Bates, 1993).

In order to overcome problems of short residual activity and to provide protection against re-infestation, a controlled-release formulation of ivermectin has been developed (Forbes *et al.*, 1999). These ivermectin boluses consist of a polypropylene cylinder with wings attached and held to the exterior of the cylinder using cellulose tape. Once inside the rumen, the tape dissolves allowing the wings to extend, therefore making the device unable to leave the reticulo-rumen. The bolus releases ivermectin at 20-40μg/kg/day and provides 100% efficacy against both established and challenge *Psoroptes* infestations. However, this is not available in the UK. Long-acting formulations of ivermectin are also available. Groups of six

calves infested with *Psoroptes* were treated with ivermectin long-acting injectable, generic long-acting ivermectin or doramectin solution. After 7-8 weeks, calves treated with long-acting ivermectin had significantly fewer mites, as detected in skin scrapings, than those treated with generic long-acting ivermectin or doramectin (Rehbein *et al.*, 2002).

The use of entomopathogenic fungi for the control of insect pests has received renewed interest amid fears of chemical pesticide resistance and environmental contamination (Gillespie and Moorhouse, 1989). Fifty eight species of fungi have been observed infecting the Acari, both naturally and experimentally (Chandler *et al.*, 2000). However, the use of fungi as biological control agents has provided little success to date, largely due to the temperature and humidity conditions required for fungal growth and the barrier that the insect exoskeleton provides to fungal infection. Development of a fungal pathogen against *Psoroptes* mites is in the early stages although some positive results have been observed. Smith *et al.* (2000) found that the fungus *Metarhizium anisopliae* (Metchnikoff) is highly pathogenic to *Psoroptes* mites with 71% of mites infected following immersion for 10 mins in a suspension of 1×10^7 conidia ml^{-1}. Brooks and Wall (2001) found infection rates of 77% when immersed for 10 mins in a 1×10^8 ml^{-1} and 73% of mites became infected after contact for 24 h with a surface treated with a fungal suspension of the same concentration. Uninfected live mites were also found to acquire fatal infections from contact with infected cadavers with five day old cadavers being the most effective in transmitting the infection. *Metarhizium anisopliae* tends to grow better at lower temperatures and the number of *Psoroptes* infections produced by the fungus declines at higher temperatures. However particular high-temperature adapted *M. anisopliae* isolates, when formulated in silicone oil, are able to produce higher levels of infection even at the relatively high

temperature of 37.5°C (Brooks *et al.*, 2004). This suggests that *M. anisopliae* is a good candidate for biological control of *Psoroptes* mites even at the conditions found on the host.

Increased attention is also being given to the use of natural products for the control of ecto-parasites, with acaricidal activity demonstrated in extracts of lavender and camomile (Perrucci *et al.*, 1996; Macchioni *et al.*, 2004). Perrucci *et al.* (1995) demonstrated acaricidal activity of several compounds that are components of plant essential oils. Mites isolated from rabbits were exposed to the compounds either by direct contact or by inhalation. Direct contact was achieved by placing groups of 10 mites into a petri dish containing the compound diluted to one of three concentrations in saline, and inhalation by placing 10 mites in a petri dish covered with a piece of filter paper to allow gas exchange, and then enclosing in a larger dish containing the test compound. Acaricidal activity of 100% was achieved as a result of direct contact and in some cases via inhalation. The compounds with the highest activity were the alcohols or phenols, therefore it was proposed that it was the oxygenated functional groups that caused the acaricial properties.

Attempts have been made to produce a vaccine against sheep scab although little success has been achieved as yet. It has been demonstrated that immunity to sheep scab can be acquired (van den Broek *et al.*, 2000). Exponential lesion growth was observed in all sheep naïve to *Psoroptes* but growth was much slower in previously infested sheep. After 2 weeks post infestation, lesion area in previously infested sheep was significantly smaller than in primary infestations.

Although relatively little is known about the allergens of *Psoroptes* mites, various studies have revealed possible candidate antigens that could be used in vaccine development (Jayawardena *et al.*, 1998; Pruett, 1999). Smith *et al.* (2002) demonstrated protective immunity following inoculation with soluble *Psoroptes*

extracts, with immunised animals having a two and seven-fold reduction in mean lesion area and mite numbers, respectively. These protective effects were confirmed by Smith and Pettit (2004) who attained a three and 13-fold reduction in mean lesion area and mite numbers, respectively. However, the polypeptide profile of the most protective fraction was still complex, suggesting further work is necessary to identify individual protective antigens.

1.7 Aims

The aims of the work described here were to examine aspects of the morphology and behaviour of *Psoroptes* mites with particular reference to their behaviour *in vitro* with a view to developing an *in vitro* colony of mites. First, because all the mites to be used in the current work were to be derived from infested rabbits, it was vital to be confident that these mites were similar to those found infesting sheep, so that any behaviour observed could be extrapolated to mites derived from sheep. As a result, a morphological comparison of host-derived populations of *Psoroptes* mites was undertaken (Chapter 3). Subsequently, to understand the behaviour of the mites off-host, the responses of the mites to light, temperature and gravity were considered (Chapter 4). Finally, the effect of various environmental conditions and diets were examined to determine optimum conditions for maximum mite longevity and which would aid the development of an *in vitro* colony greatly (Chapter 5).

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 Mite colony

Psoroptes mites were reared in the ears of two New Zealand white rabbits. An *in vivo* culture of these mites has been maintained at the University of Bristol for approximately eight years. Rabbits were replaced approximately every two years. The infestations were transferred to new rabbits either by anaesthetising the animal and gluing a piece of scab containing mites to the inner ear or by placing some scab and mites in the pinna of the ear and holding it closed using adhesive tape. The mite colony was maintained under Home Office Licence number PPL 30/1636 until September 2004 and PPL 30/2205 thereafter.

2.2 Collection of mites

Auricular infestation results in the leakage of serous exudates which dry to form scabs on the inner surface of the pinnae of the ears. Throughout this work, efforts were taken to maintain as small an infection as possible in the rabbits to minimise any pathological effects or welfare problems. To do this, twice weekly, the rabbits were restrained and the scabs carefully removed using blunt ended forceps. The scabs and mites present were placed into a clean plastic container, 40 mm in diameter and 60 mm high. Once removed from the host, the mites migrate out of the scab and up the sides of the plastic container from which they were removed using a fine paintbrush.

2.3 Mite identification

Once removed from the scab, the various mite life-cycle stages needed for experiments were identified using a binocular dissecting microscope. Identification of adult female mites was determined primarily by their relatively large size and confirmed by the presence of an inverted U-shaped vulva on the ventral surface of the mite (Fig. 2.1). Adult male mites were identified by the presence of the gonopore (reproductive apparatus) and adanal suckers and by the fact that leg IV is much shorter than all other legs (Fig. 2.2). Female protonymphs were distinguished by their body shape, being relatively broad at the anterior idiosoma and narrow at the point where the podosoma and opisthosoma join, and by the presence of dorsoposterior tubercles (Fig. 2.3a). Dorsoposterior tubercles are also present on female tritonymphs, but they can be distinguished from the female protonymphs by the absence of a pre-tarsus or pulvillus on leg IV (Fig. 2.3b). Male nymphs were identified by the absence of dorsoposterior tubercles with the tritonymph being larger in size than the protonymph (Figs. 2.4a & b). Larvae, which show no sexual dimorphism, were identified as being the smallest of all the stages and having just three pairs of legs compared to the four pairs seen in all other life-cycle stages (Fig. 2.5).

Figure 2.1 Ventral view of adult female *Psoroptes ovis* mite showing the vulva, the main morphological feature used for life-cycle stage identification. (Adapted from Sanders *et al.*, 2000).

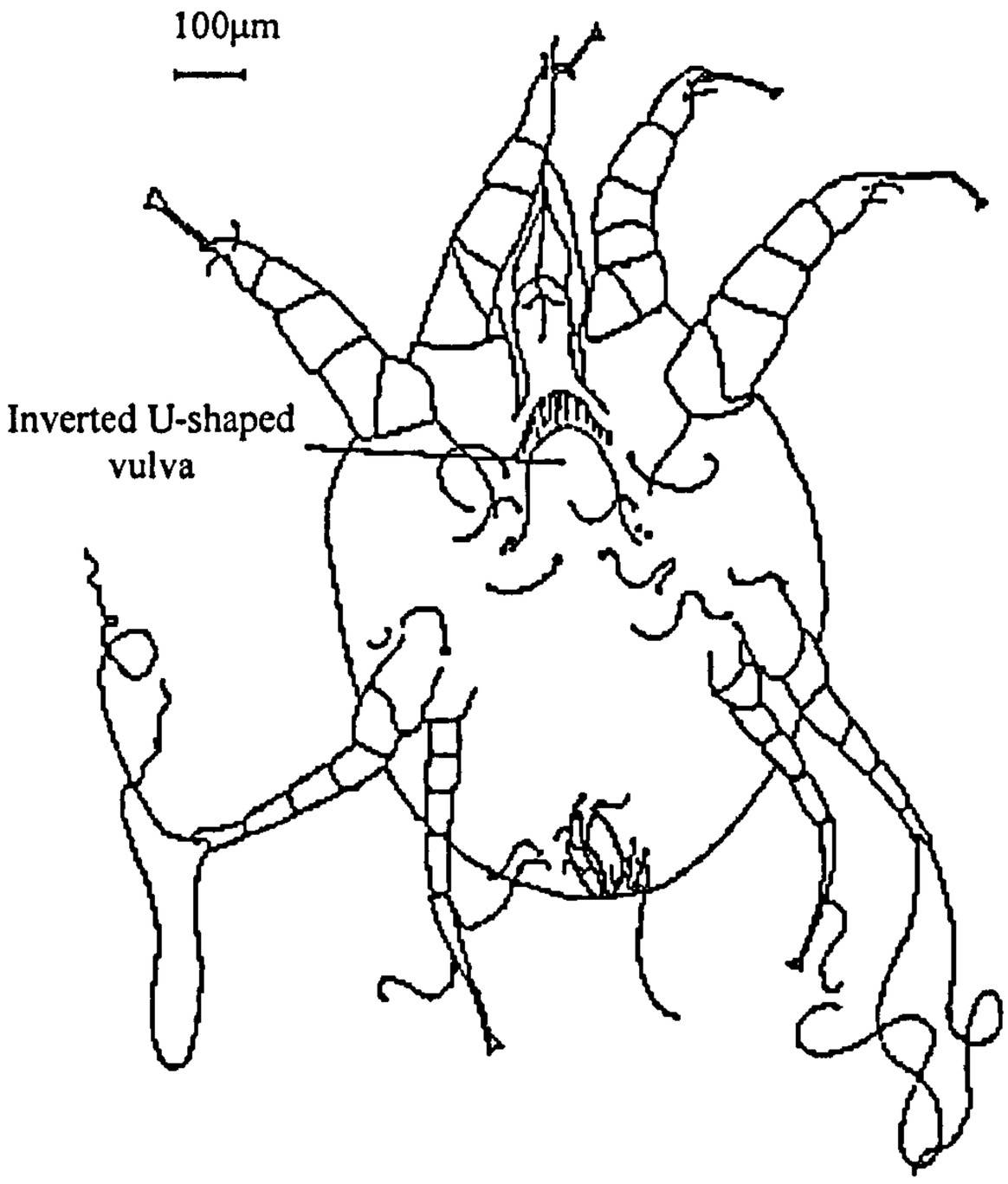


Figure 2.2 Ventral view of adult male *Psoroptes ovis* mite showing the gonopore, the adanal suckers and the shortened leg IV, the main morphological features used for life-cycle stage identification. (Adapted from Sanders *et al.*, 2000).

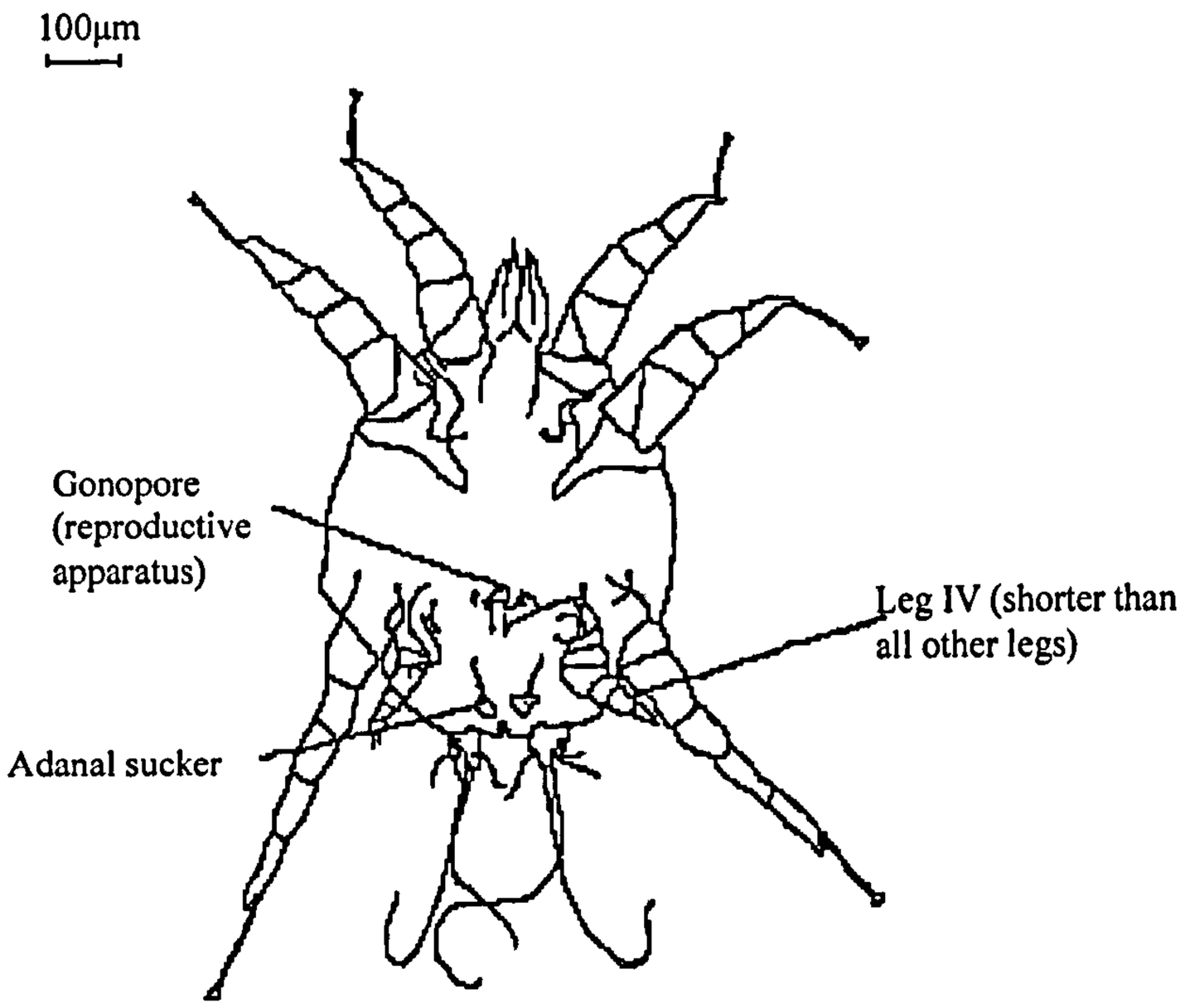


Figure 2.3 Dorsal view of female nymphs of *Psoroptes ovis.* (a) protonymph, showing the dorsoposterior tubercles and distinctive body shape, (b) tritonymph, showing the dorsoposterior tubercles and leg IV lacking a pretarsus or pulvillus, the main morphological features used for life-cycle stage identification. (Adapted from Sanders *et al.*, 2000).

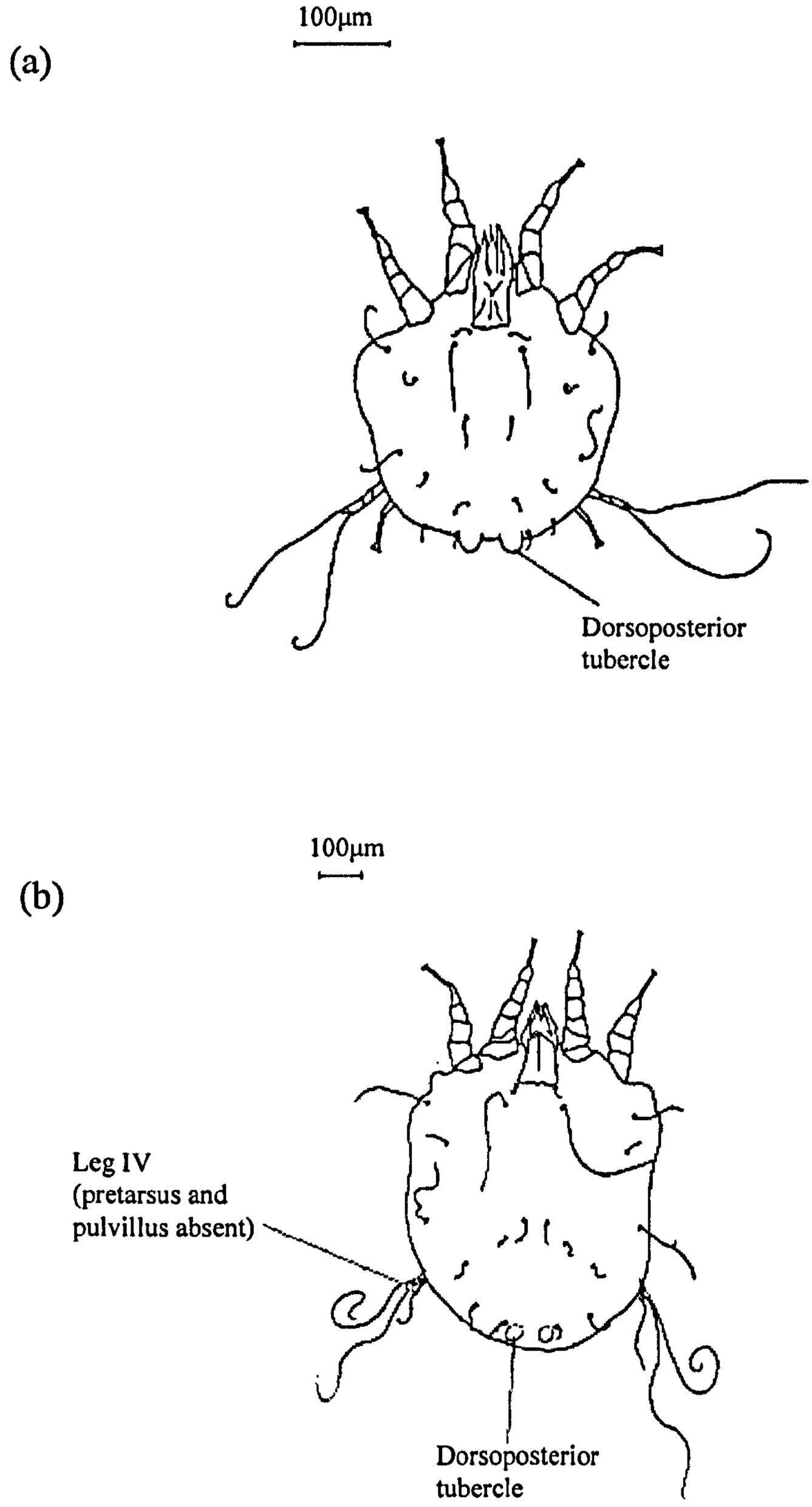


Figure 2.4 Dorsal view of male nymphs of *Psoroptes ovis,* both nymphal stages lack dorsoposterior tubercles with the protonymph (a), being smaller in size than the tritonymph (b). (Adapted from Sanders *et al.* (2000)).

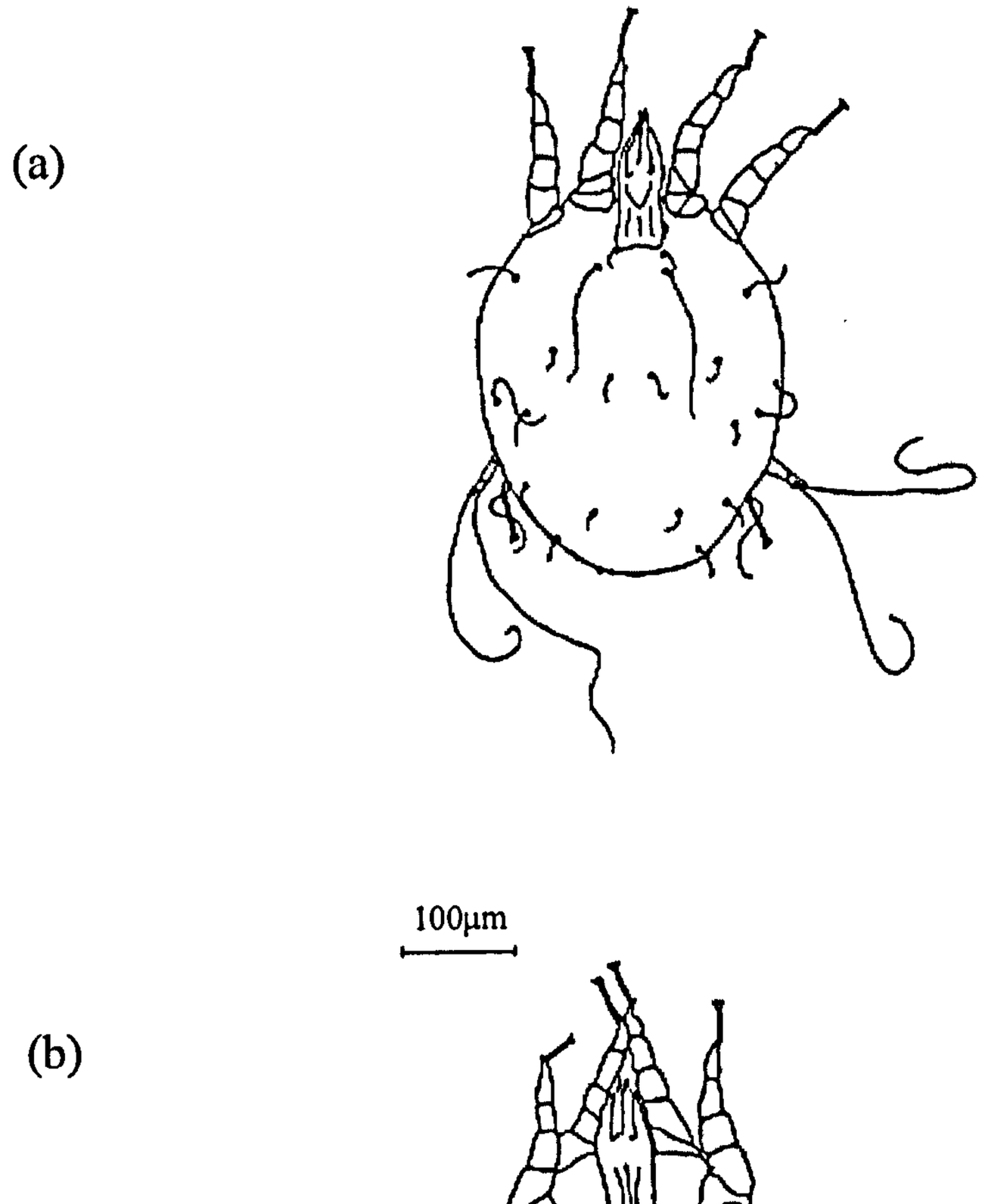
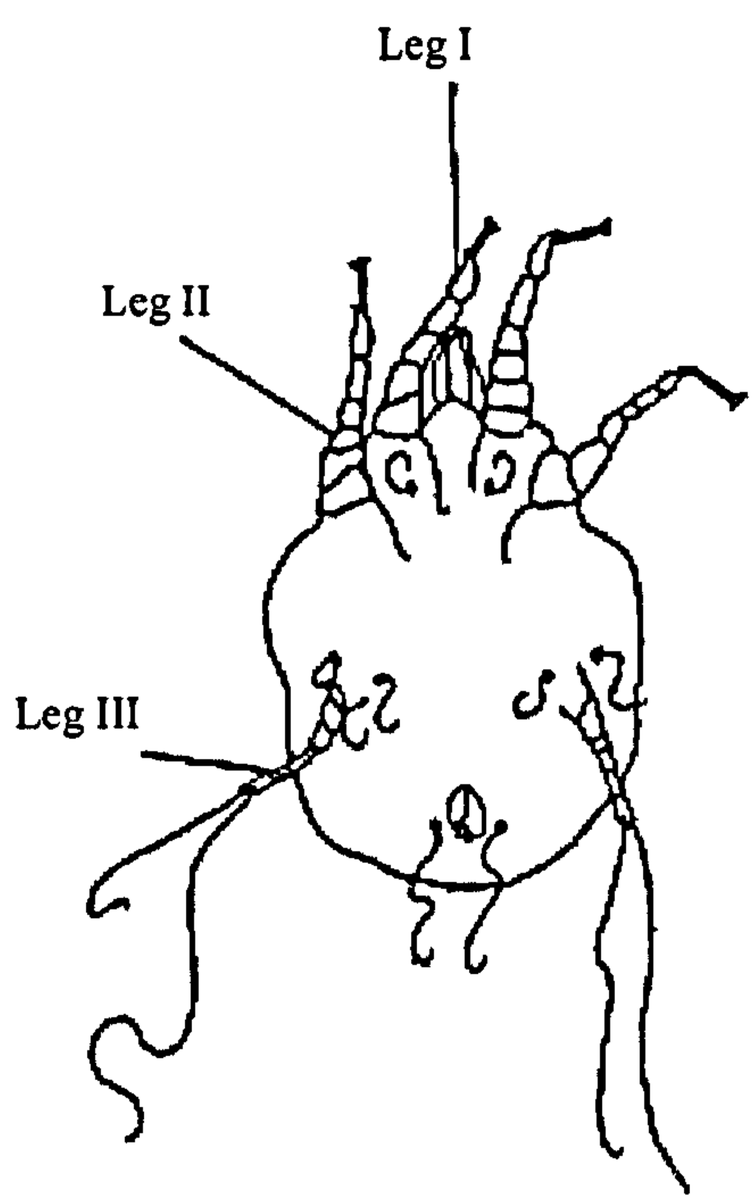


Figure 2.5 Ventral view of larva of *Psoroptes ovis,* showing three pairs of legs, the absence of a fourth pair being the main morphological feature used for life-cycle stage identification. (Adapted from Sanders *et al.*, 2000).



CHAPTER 3

MORPHOLOGICAL COMPARISON OF HOST-DERIVED POPULATIONS OF *PSOROPTES* MITES

3.1 Introduction

Mites of the genus *Psoroptes* are distinguished by the presence of a terminal sucker on a relatively long, three segmented pre-tarsus (Hirst, 1922). The most well known and clinically important occurrence of this mite is on sheep, and the causative agent was described and first given the name *Sarcoptes ovis* by Hering in 1838, but later assigned to the genus *Psoroptes*. Following the initial description from sheep, as many as nine species of *Psoroptes* mite were proposed, each distinguished from the others mainly by the different mammalian hosts they infest. However, because of the morphological similarity between mites found on different host species, a number of early authors believed that most populations on different hosts belonged to a single species, synonomised initially as *Dermatokoptes communis* by Fürstenberg in 1861 and subsequently as *Psoroptes communis*. Host-derived populations were referred to as *P. communis* with varietal names such as *ovis* or *bovis* appended, depending on the host species from which they were obtained (Raillet, 1893; Stockman & Berry, 1913; Shilston, 1915).

In 1958, Sweatman carried out a detailed study in an attempt to find a stable morphological character which could be used to separate putative species of the genus *Psoroptes*. From all of the measurements taken, he proposed that only the outer opisthosomal setae of adult males could be used to distinguish, what were regarded by Sweatman, as five true species of *Psoroptes*. These included *P. ovis*

from sheep and *Psoroptes cuniculi* found in the ears of domestic rabbits. This classification has achieved general acceptance in the literature (Strong and Halliday, 1992). However, use of this taxonomy has proved difficult, largely due to the high degree of variance in setae length within populations from any individual host and populations from different hosts (Lange *et al.*, 1980; Wright *et al.*, 1984; Bates and Sayers, 2002).

All of the previous studies of the morphological characters of *Psoroptes* have examined adult male mites only and few of these studies actually support Sweatman's five putative species. Wright *et al.* (1984) compared outer opisthosomal seta lengths of adult male mites from a range of hosts and found considerable overlap and also a significant difference in outer opisthosomal seta length between mites from the same hosts that Sweatman's key would place as the same species. They suggested that outer opisthosomal length did not have sufficient reliability to form the basis of a taxonomy. A comparison of outer opisthosomal seta lengths on adult male mites was also carried out by Bates and Sayers (2002). Although some significant differences in outer opisthosomal setae length were found between populations of mites from different hosts, a large degree of overlap was observed thus it was concluded that mites from sheep, goats, cattle, rabbits and alpaca are variants of the same species.

The idea that there might in fact be only a single species of *Psoroptes* was also supported by host transfer experiments, some of the first of which were carried out by Sweatman himself. Sweatman successfully transferred ear mites from a rabbit to a goat and from a rabbit and a goat to a sheep. Meleney (1967) successfully transferred body mites from cattle to a rabbit and then back to cattle and sheep, and Wright (1982) successfully reared mites from the ears of rabbits on cattle and found that the mites were still infective to rabbits after 20 generations on cattle.

One report suggests that *P. ovis* and *P. cuniculi* have been successfully cross mated in the ears of rabbits to produce viable offspring (Wright *et al.*, 1983). Both species were found to be infective to rabbits and the hybrid mites were found to be infective to calves. Outer opisthosomal seta lengths were found to be significantly different between the two parent populations but within the hybrid population, lengths did not differ significantly between individual mites and were found to be within the parental range. Although this provides strong supportive evidence that there is in fact a single species of *Psoroptes*, important details are lacking in this paper, for example the precise origin of the two parent mite populations is not stated. Up until now, cross mating has not been successfully repeated or confirmed.

During host transfer experiments some differences in the extent of lesion growth and severity of infection have been noted (Meleney, 1967; Wright, 1982; Meintjes *et al.*, 2002b), and attributed to the presence of the different populations of *Psoroptes* present. For example Roberts and Meleney (1971) noticed what they considered to be two different strains of mites infesting sheep, an avirulent strain where mite numbers reduced greatly in summer but rose sharply in winter and a virulent strain where mite numbers and clinical signs did not reduce in the summer months and where mites were found to be more resistant to dipping with organophosphates.

Meintjes *et al.* (2002b) noted faster lesion growth on Merino sheep than Dorper sheep when infested with mites taken from Merino sheep. It has been suggested that outer opisthosomal seta length might be associated with virulence and could be used as an indicator of virulence (Bates and Sayers, 2002).

Genetic and antigenic studies also largely support the idea of a single *Psoroptes* species. Zahler *et al.* (1998) examined the second internal transcribed spacer region (ITS-2) of the ribosomal RNA gene, a region which has proven to be useful for distinguishing between species in acarid genera, and found mite isolates to

be genotypically highly homogeneous. Phylogenetic analysis of DNA sequence data from the first internal transcribed spacer region (ITS-1) of nuclear ribosomal DNA has revealed *Psoroptes* populations not to be host specific (Ramey *et al.*, 2000). However Ochs *et al.* (1999) found a single nucleotide difference in the ITS-2 region of rDNA which they suggested could be used to distinguish between mites from sheep and rabbits. Boyce and Brown (1991) found only minor antigenic differences between mites from cattle, rabbits and bighorn sheep, but found mites of mule deer to be antigenically identical to those of bighorn sheep. Host transfer experiments of mites between rabbits and sheep have also shown that these mites are antigenically cross-reactive (Siegfried *et al.*, 2004).

The aim of the work described in this chapter therefore was to determine whether outer opisthosomal seta length is indeed the most stable character for distinguishing between mites of different host species or host-derived populations and whether this morphological approach can also be used to distinguish between adult female mites from different hosts.

3.2 Materials and methods

Dead adult male and female *Psoroptes* mites, stored in ethanol, were cleared in Nesbitt's fluid (chloral hydrate 40g, distilled water 25ml, HCl conc. 2.5ml) for approximately 3 days before mounting, dorsal side uppermost, in Hoyer's medium (chloral hydrate 200g, distilled water 50ml, gum arabic 30g, glycerine 20ml). The preparation was dried at approximately 25°C for 3 days and the edge of the glass coverslip sealed with clear nail varnish.

Specimens were examined under a microscope and digital photographs taken. Measurements of morphological features were taken using a computer programme (Leica Qwin, Leica Imaging Systems Ltd, Cambridge, UK); nine measurements were taken from female mites and eight from male mites (Fig. 3.1). The characters

measured were outer opisthosomal seta length; body length; body width 1, width of body measured at the base of leg II; body width 2, width of body at base of leg III; leg III posterior seta length (measured in adult female mites only); gnathosoma length; gnathosoma width; length of ambulacrum of leg I; propodosomal seta length (pair below vulva on female). Where characters are found in pairs, (outer opisthosomal setae, leg III posterior setae, ambulacrum of leg I, propodosomal setae), both characters were measured and the mean value was determined. Preliminary analysis had shown that the left and right characters did not differ significantly. Where it was only possible to make one measurement, this value was used alone.

Samples of mites were collected or provided from various host species and from various geographical locations worldwide (Table 3.1). In most cases the mites were supplied without detailed notes relating to their provenance, only the host species from which they were collected.

Figure 3.1. Morphological characters measured on (a) adult female (dorsal and ventral view) and (b) adult male (dorsal and ventral view) mites. (Adapted from Sanders *et al.*, 2000)

Table 3.1. Isolate, provenance, sex and number of *Psoroptes* mites examined.

Host species	Host location	No. males	No. females	Location on host	Notes
Domestic rabbit	Bristol, UK	48	45	Ear	University of Bristol
	Bristol, UK	9	4	Ear	
Sheep	Yorkshire, UK	9	10	Body	
	Scotland, UK	3	5	Body	
	Gloucestershire, UK	2	0	Body	
	Scotland, UK	6	0	Body	
	Lancashire, UK	6	3	Body	
	Wales, UK	0	12	Body	
	Lancashire x Cornwall, UK	3	10	Body	VLA Strain
	York, UK	0	5	Body	
	Devon, UK	0	1	Body	
	Cornwall, UK	4	3	Body	
	Cumbria, UK	0	1	Body	
	Scotland, UK	1	3	Body	
	Yorkshire, UK	2	1	Body	
	Dublin, Ireland	1	1	Body	'88 strain – isolated since '86
	Chester, UK	1	6	Body	
	Wales, UK	0	5	Body	
	Powys, Wales, UK	1	4	Body	
	Gwynedd, Wales, UK	1	3	Body	
	Powys, Wales, UK	0	1	Body	
	Wales, UK	1	6	Body	
	SW England, UK	3	4	Body	
	Bloemfontein, South Africa	8	18	Body	Provided by Prof. L. Fourie
Alpaca	Chile, South America	1	1	Ear	
	Chile/Bristol	8	7	Ear	Animal imported into UK from Chile; source of infestation unknown
Goat	Bristol, UK	2	1	Unknown	
	Georgia, USA	6	0	Unknown	
Cow	Bristol, UK	14	18	Unknown	
	Belgium	7	29	Body	Provided by Dr. M. Lekimme.
Big horn sheep	New Mexico, USA	7	6	Unknown	
	New Mexico, USA	5	8	Unknown	
	Utah, USA	1	0	Unknown	
Mule deer	New Mexico, USA	6	6	Unknown	
Elk	Idaho, USA	6	1	Unknown	
White tailed deer	USA	5	5	**Unknown**	
	Total	**177**	**233**		

Analysis

Initial analysis considered autocorrelation between the characters measured. As a result, the number of morphological characters used in the analysis was reduced to five in males and six in females to avoid correlation between characters. A discriminant analysis (SPSS 12.0, SPSS Inc. Chicago, USA) with host species as the grouping variable was used to determine linear combinations of morphological characters that could be used to distinguish between mites infesting the various host species. This analysis was carried out first for both adult male and female mites for samples with more than ten mites, pooling isolates from the same species of host. It was then repeated for all isolates, regardless of sample size, but again pooling isolates from the same species of host.

The measurements of the characters identified by the discriminant analysis as most highly related to host species differences, were compared separately using a one-way ANOVA, first for samples of greater than ten mites pooling isolates from the same species of host and repeated with all isolates regardless of sample size, but again pooling isolates from the same species of host. Subsequently, ANOVA and Tukey HSD post-hoc tests or t-tests were used to consider differences in character measurements between individual isolates within the same host species. The normality of the data was confirmed using a 1-sample K-S test and Levene's test was used to check for homogeneity of variances.

3.3 Results

Adult males: more than 10 mites: pooled isolates

When the discriminant analysis was carried out using only samples where there were more than ten mites, three linear functions of the five morphological features were able to explain a significant proportion of the variance in host species. Function 1, with an eigenvalue of 4.76, explained 87.9% of the total variance. Function 1 was most highly correlated with outer opisthosomal seta length. The correlation between function 1 and outer opisthosomal seta length was 0.95. Functions 2 and 3 were most highly correlated with gnathosoma length and propodosomal seta length and these explained 8.0 and 4.1% of the variance respectively. When functions 1 and 2 are plotted against each other, the mites from rabbits, sheep, cattle and bighorn sheep are seen to be clearly separated into groups (Fig. 3.2).

Outer opisthosomal seta length differed significantly between mites from bighorn sheep, cattle, rabbits and sheep ($F_{3,139}$=198.24, P<0.001)(Fig. 3.3). The setae were longest in cattle and shortest in rabbits.

Gnathosoma length was the second most important function identified by the discriminant analysis for distinguishing between adult male mites from different hosts when only sample sizes of greater than 10 were considered. Overall gnathosoma length does differ significantly between mites from different hosts ($F_{3,139}$=12.55, P<0.001) but Tukey post-hoc tests revealed that the gnathosoma length of mites from cattle, sheep and bighorn sheep do not differ significantly from each other (Fig. 3.4).

Figure 3.2. Two linear functions of five morphological characters measured on 143 adult male *Psoroptes* mites as revealed by discriminant analysis. Functions 1 and 2 are most highly correlated with outer opisthosomal seta length and gnathosoma length respectively.

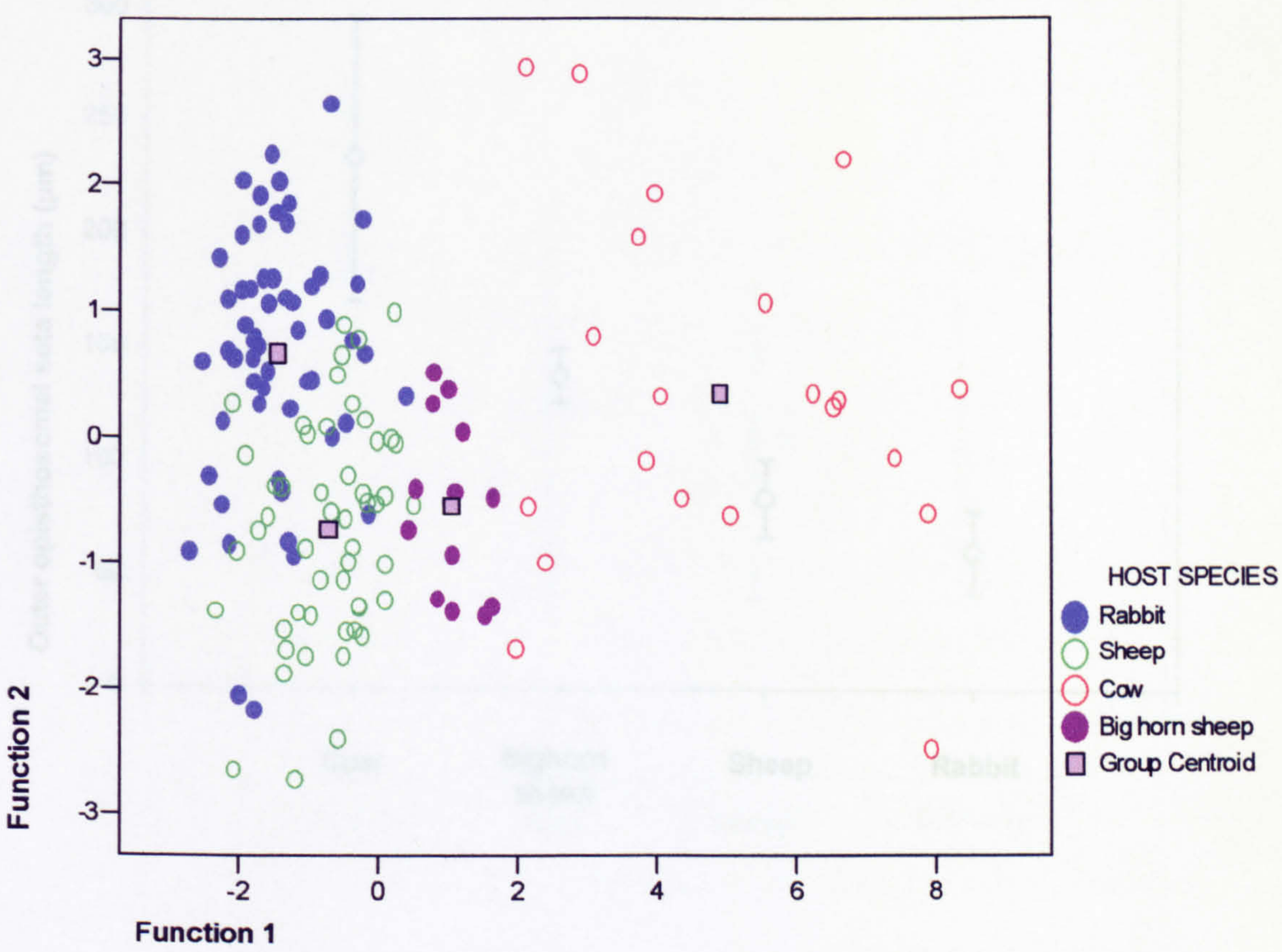


Figure 3.3. Mean outer opisthosomal seta length (µm ±s.d.) of adult male *Psoroptes* mites collected from various host species. All points are statistically significantly different from each other ($F_{3,139}$=1928.24, P<0.001).

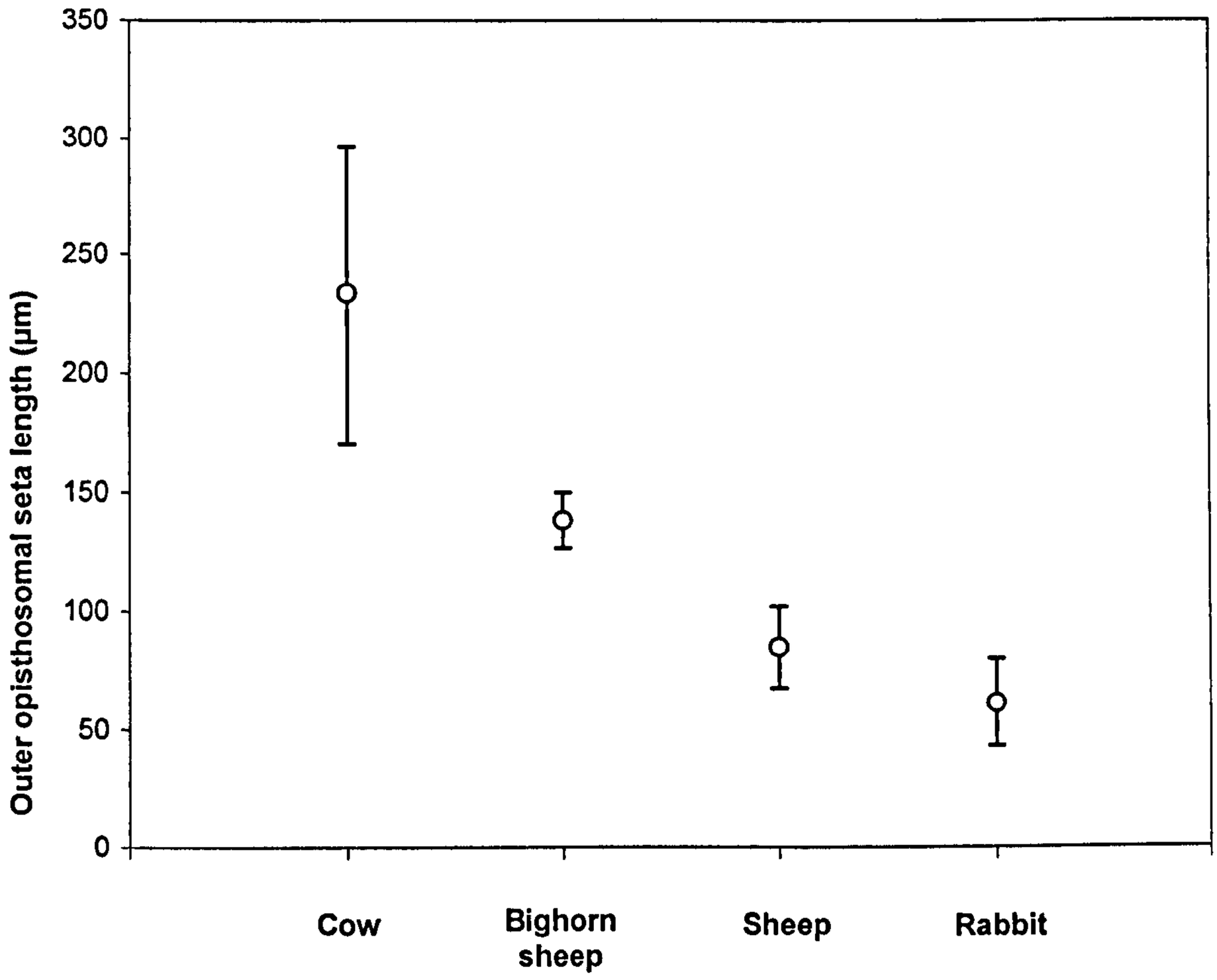
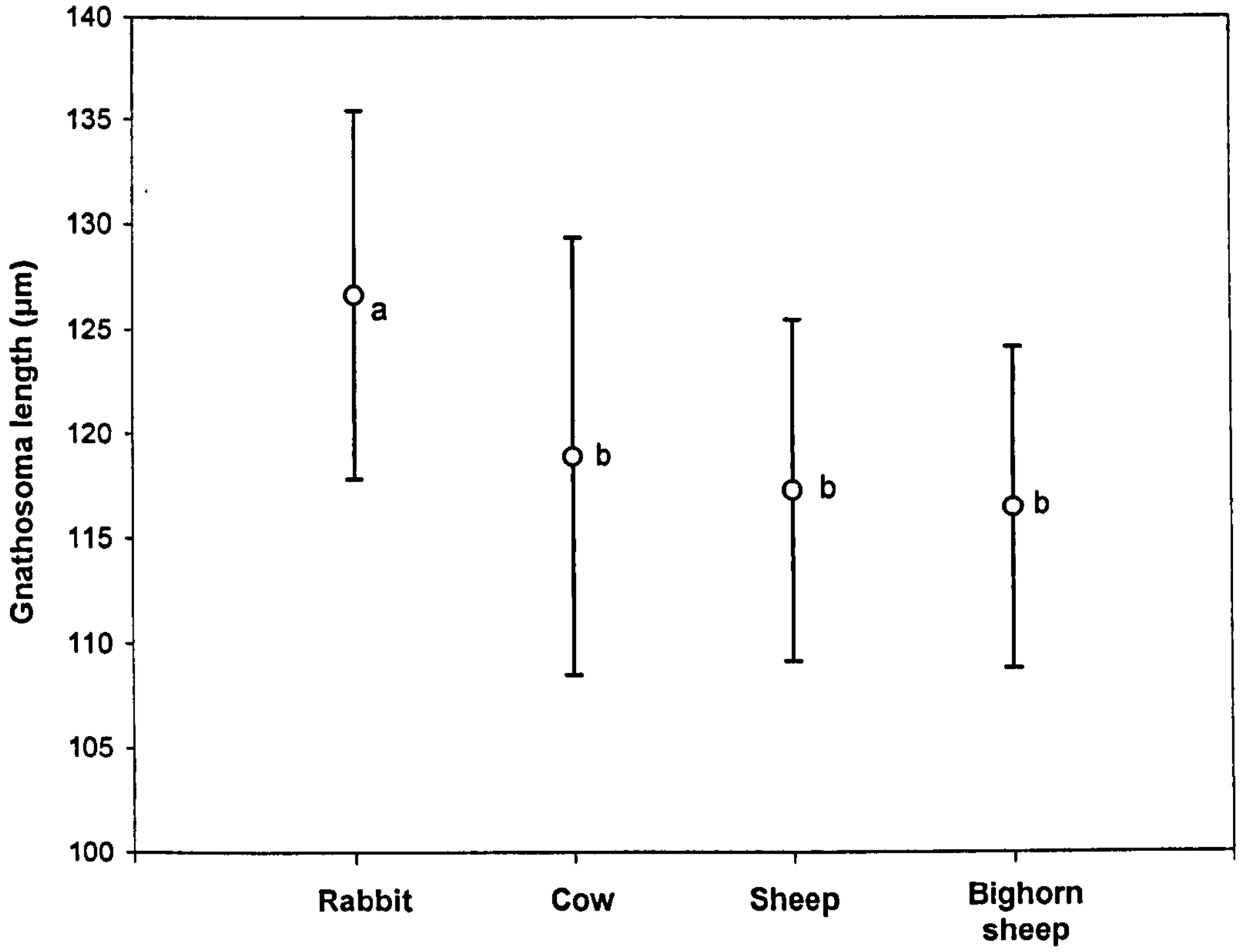


Figure 3.4. Mean gnathosoma length (μm ±s.d.) of adult male *Psoroptes* mites of different host species. Letters indicate points between which there is no statistically significant difference ($F_{3,139}$=12.55, P<0.001).



Adult males: all samples: pooled isolates

When the discriminant analysis was repeated using mite samples from all host species, five significant linear functions were revealed. Function 1, with an eigenvalue of 4.03, was again most highly correlated with outer opisthosomal seta length with a correlation coefficient of 0.95. Function 1 explained 74.3% of the total variance and the first three functions combined explained 96.2% of the variance. Functions 2 and 3 were most highly correlated with body width and gnathosoma length and explained 12% and 9.9% of the variance, respectively. When functions 1 and 2 are plotted against each other, grouping of the mite samples by host can be seen although there is less distinction than in the previous analysis, particularly of mites from elk and cattle and from big horn sheep and alpaca (Fig. 3.5).

There is an overall significant difference between the outer opisthosomal seta lengths of mites from different host species. Again, the setae of cattle mites are the longest and rabbit mite setae are the shortest of those measured ($F_{8,168}$=76.46, P<0.001). However, post-hoc tests revealed groups within which there is no significant variation. (Fig. 3.6). Very little variation was seen in the outer opisthosomal seta lengths of mites from elk, bighorn sheep, alpaca, mule deer, white-tailed deer, sheep and goat. The outer opisthosomal seta lengths of rabbit mites were significantly different from the mites of all hosts other than goat mites. The outer opisthosomal seta lengths of cattle were significantly different in length to the mites of all other host species.

Figure 3.5. Two linear functions of five morphological characters measured on 177 adult male mites as revealed by discriminant analysis. Functions 1 and 2 are most highly correlated with outer opisthosomal seta length and body width respectively.

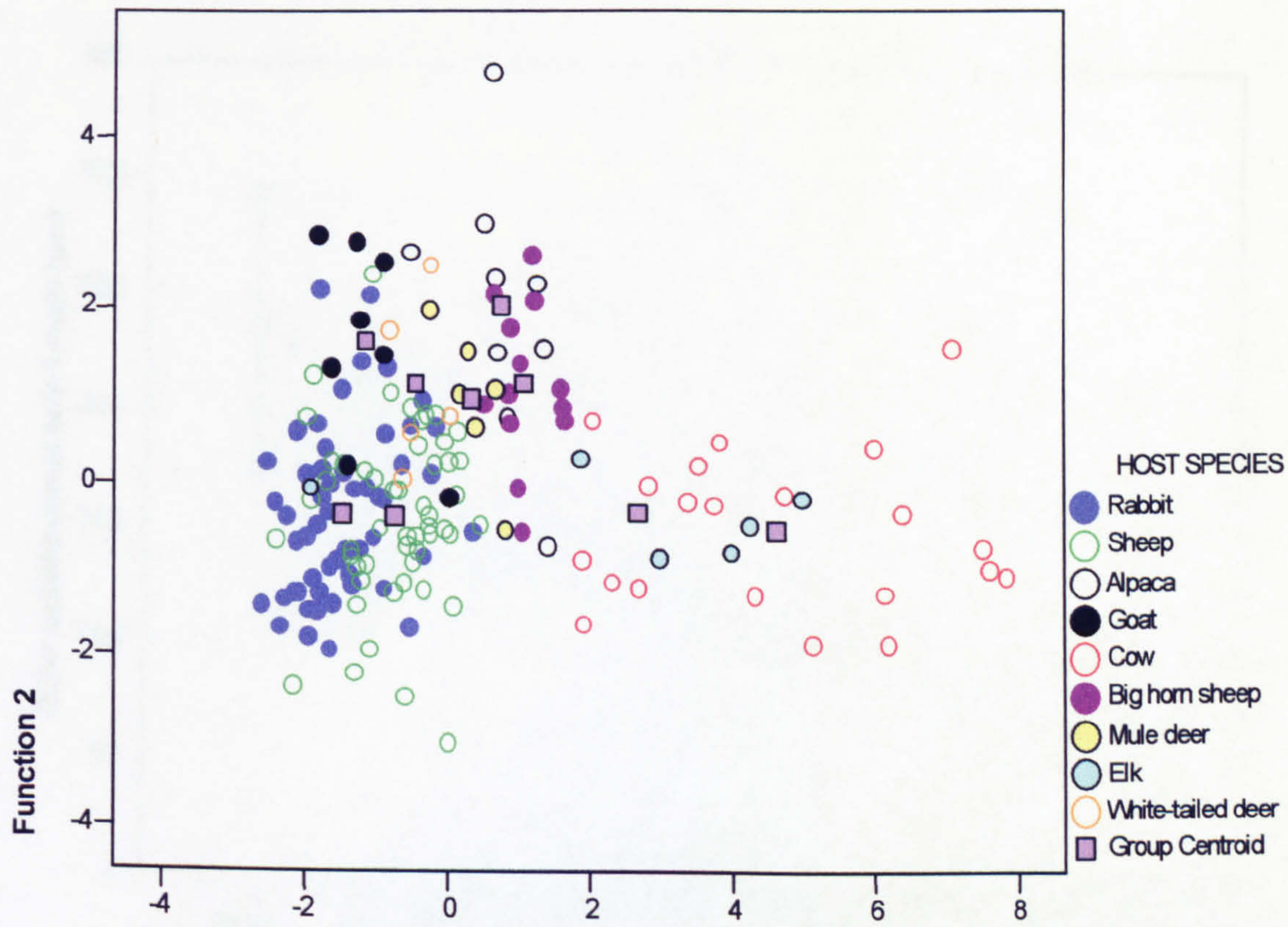
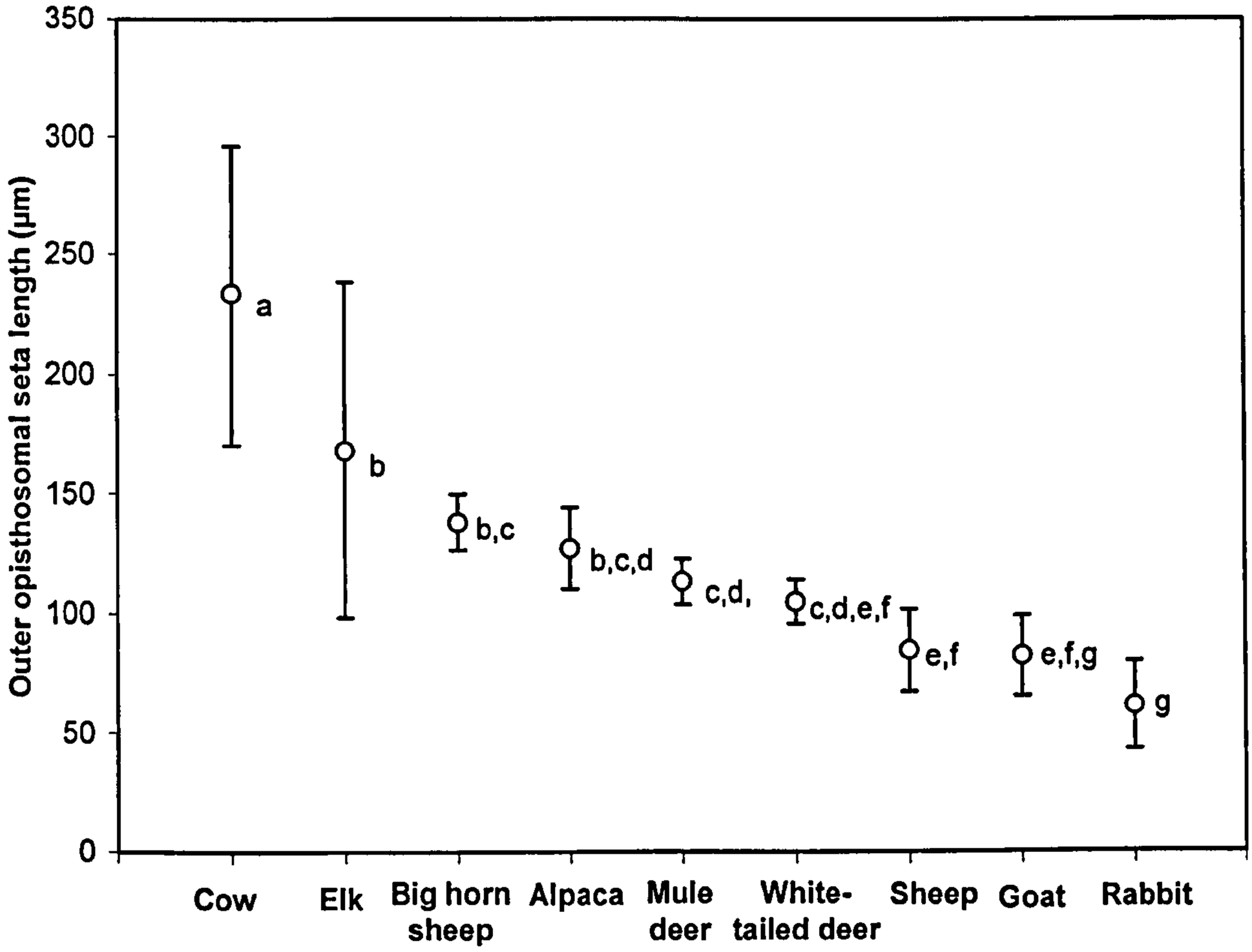


Figure 3.6. Mean outer opisthosomal seta length (μm ±s.d.) of adult male *Psoroptes* mites collected from a range of host species. Letters indicate points between which there is no statistically significant difference ($F_{8,168}$=76.46, P<0.001).



Mite body width was the second most important character for discriminating between adult male mites when all of the mite samples were included in analysis. Body width was found to be greatest in goat mites and smallest in mites of elk and overall there was a significant difference between body widths of mites from different hosts ($F_{8,168}$=14.67, P<0.001). Post-hoc tests showed that there were a number of groups comprised of mites from more than one host species between which outer opisthosomal seta length did not differ significantly (Fig. 3.7).

Adult males: all samples: individual isolates

When outer opisthosomal seta lengths are considered for individual isolates it can be seen that there is a relatively large amount of variation between mites from the same host species (Fig. 3.8). There was a significant difference between the two cattle isolates from USA and Belgium (t_{19}=-4.23, P<0.001) and between the two rabbit isolates, both from Bristol (t_{55}=5.97, P<0.001). There was a significant difference between outer opisthosomal seta lengths of mites from different sheep isolates ($F_{15,36}$=3.10, P=0.003). Post-hoc tests revealed that these differences lie between the South African and a Yorkshire and Scotland isolate. No further significant differences occur between mites from any of the other sheep isolates. For all other host species, there was no significant difference between outer opisthosomal seta lengths of individual mite isolates.

Figure 3.7. Mean body width (μm ±s.d.) measured at widest point of body for male *Psoroptes* mites from various host species. Letters indicate points between which there is no statistically significant difference ($F_{8,168}$=13.83, P<0.001).

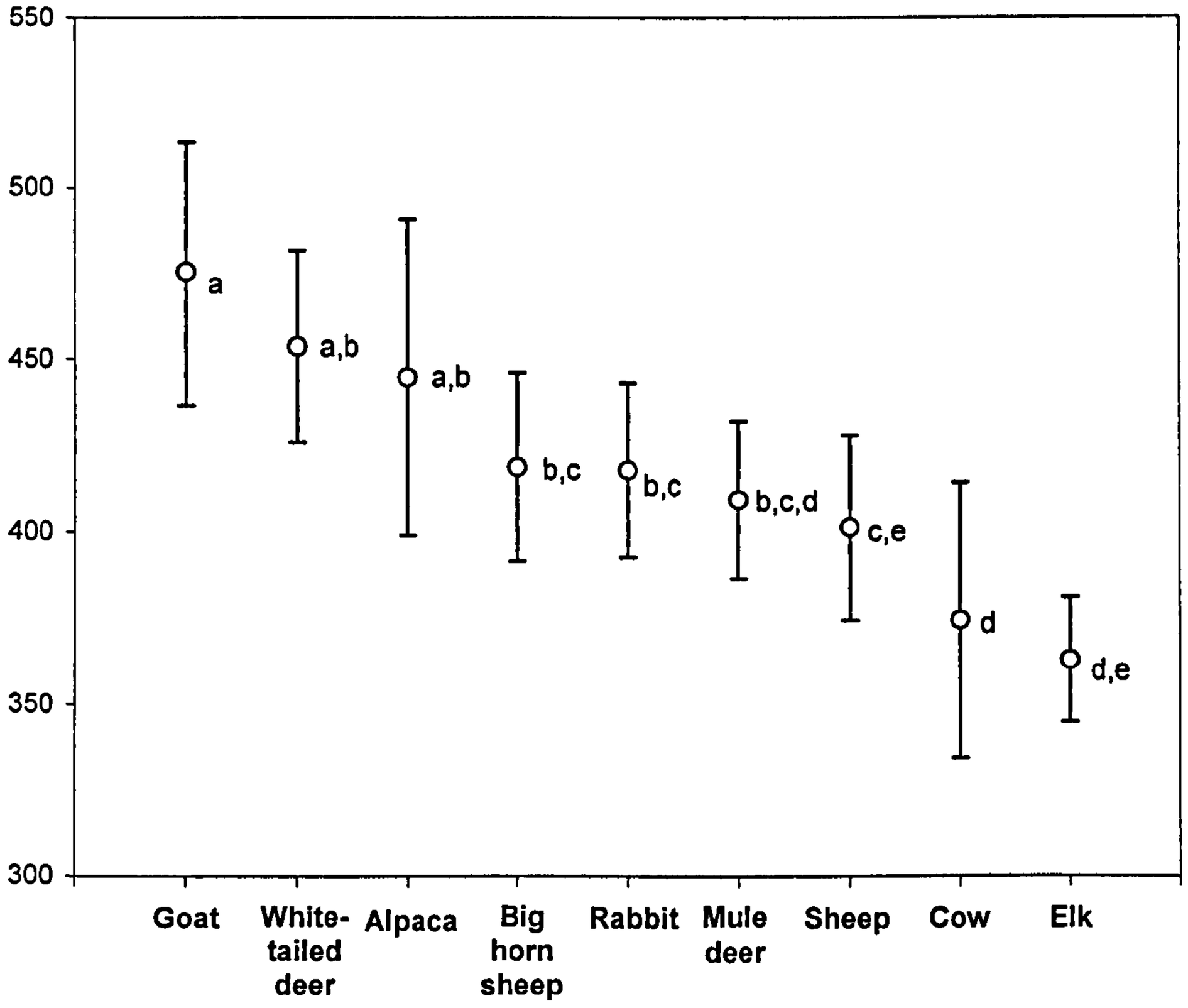
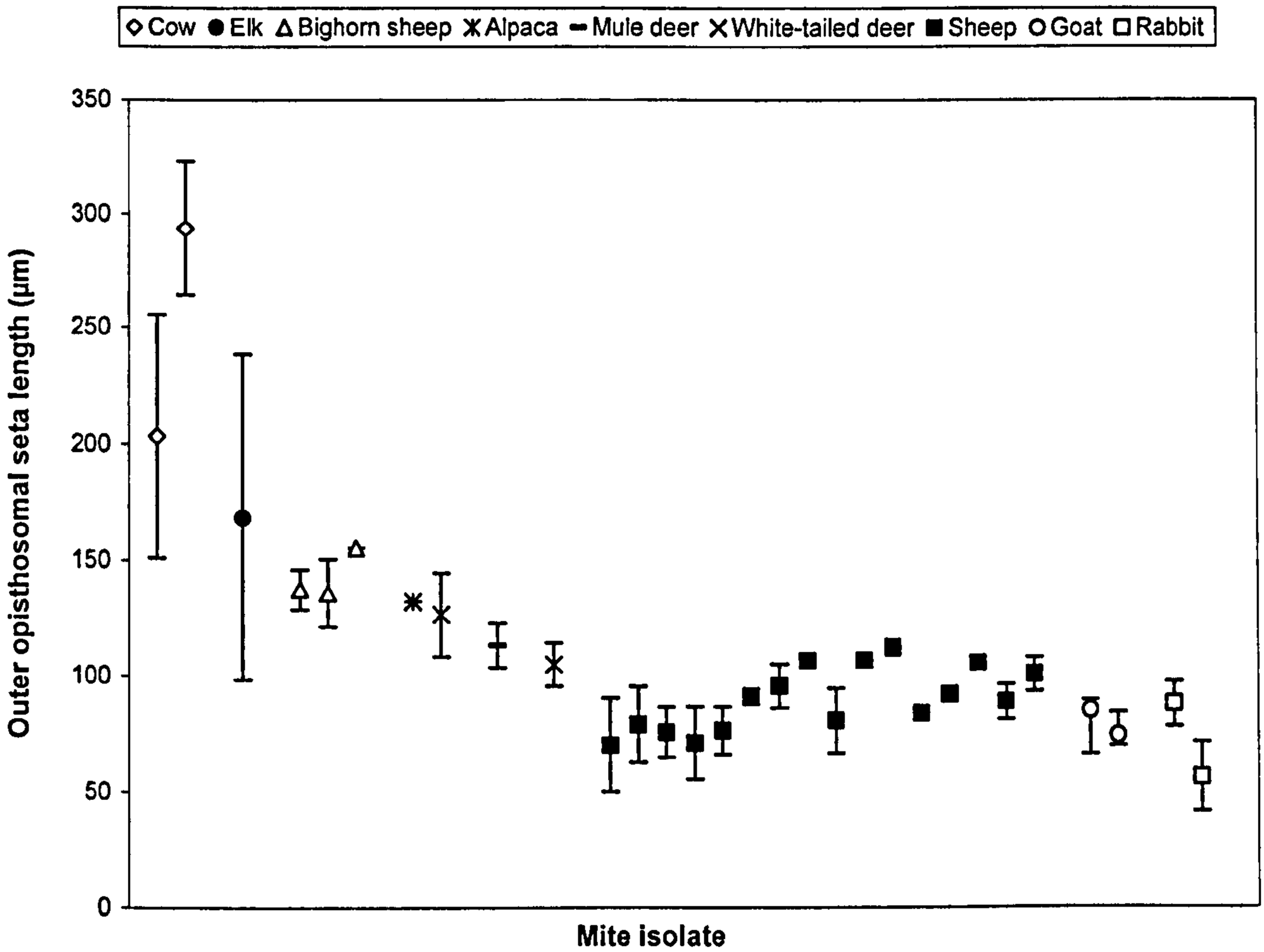


Figure 3.8. Mean outer opisthosomal seta length (μm ±s.d.) of adult male *Psoroptes* mites of all isolates examined.



Adult females: more than 10 mites: pooled isolates

When carried out on samples of adult female mites where sample sizes were greater than 10, the discriminant analysis revealed three significant linear functions. Function 1, with an eigenvalue of 2.18, explained 84.2% of the total variance and was most highly correlated with outer opisthosomal seta length, with a correlation of 0.82 between function 1 and outer opisthosomal seta length. Functions 2 and 3 were most correlated with gnathosoma length and leg 1 ambulacrum length and explained 10.9 and 4.9% of the total variance respectively. When functions 1 and 2 are plotted, the mite samples can again be seen to be separated into host species groups (Fig. 3.9).

The length of the outer opisthosomal seta of adult female *Psoroptes* mites was significantly different between mites from the different hosts ($F_{3,208}$=101.99, P<0.001), with the setae of mites from cattle and rabbits being significantly different from each other and from those of sheep and bighorn sheep. (Fig. 3.10).

The second most important function identified by the discriminant analysis for distinguishing between adult female mites from rabbits, sheep, cattle and bighorn sheep was gnathosoma length. This was found to be longest in mites of rabbits and shortest in mites of cattle and varies significantly between hosts ($F_{3,208}$=30.42, P<0.001). Tukey post-hoc tests revealed that the gnathosoma length of mites from rabbits and bighorn sheep are not significantly different from each other but do differ from those of mites of sheep and cattle (Fig. 3.11).

Figure 3.9. Two linear functions of six morphological characters measured on 212 adult female *Psoroptes* mites as revealed by discriminant analysis. Functions 1 and 2 are most highly correlated with outer opisthosomal seta length and gnathosoma length respectively.

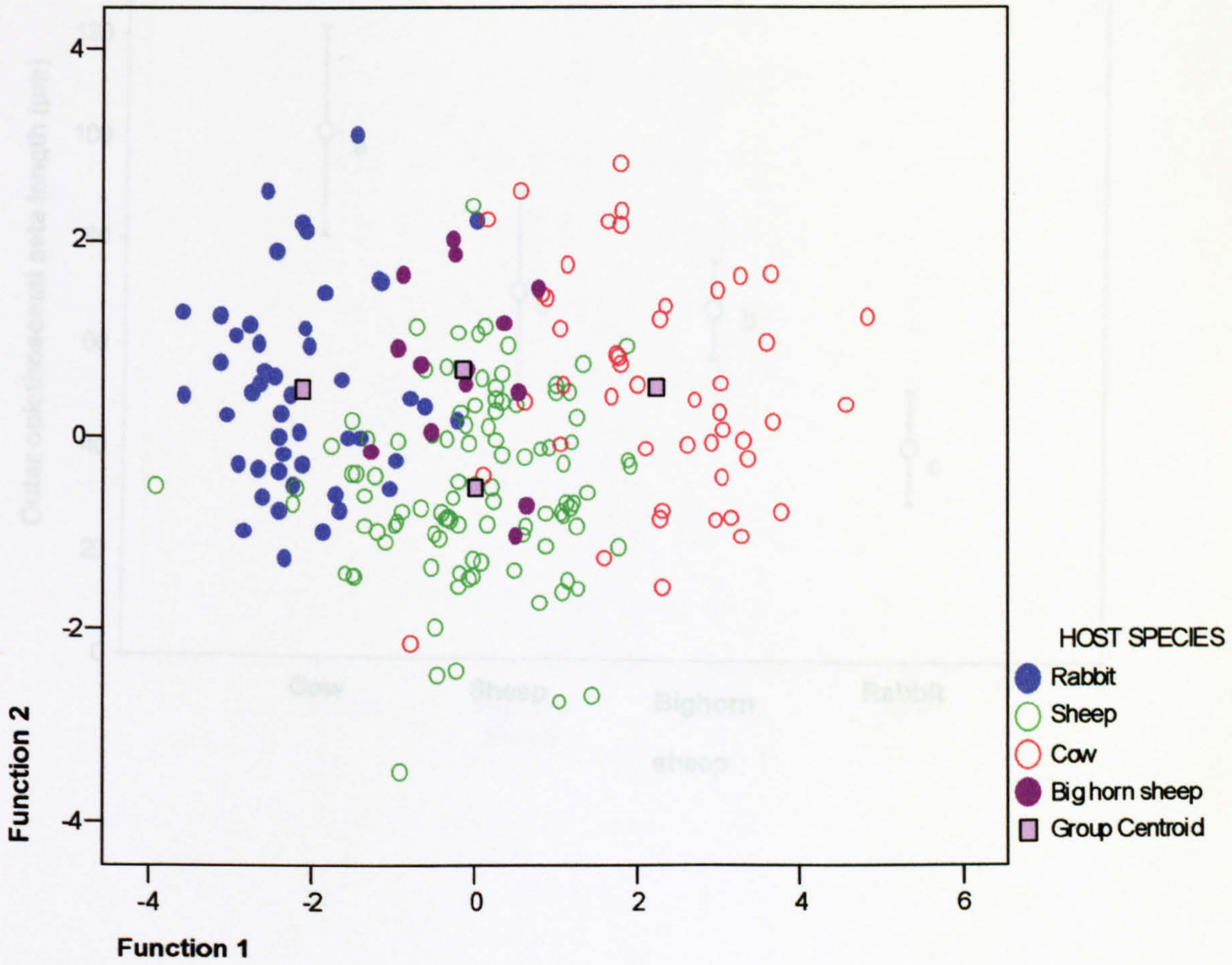


Figure 3.10. Mean outer opisthosomal seta length (µm ±s.d.) of adult female *Psoroptes* mites collected from various host species. Letters indicate groups between which there is no statistically significant difference ($F_{3,208}$=101.99, P<0.001).

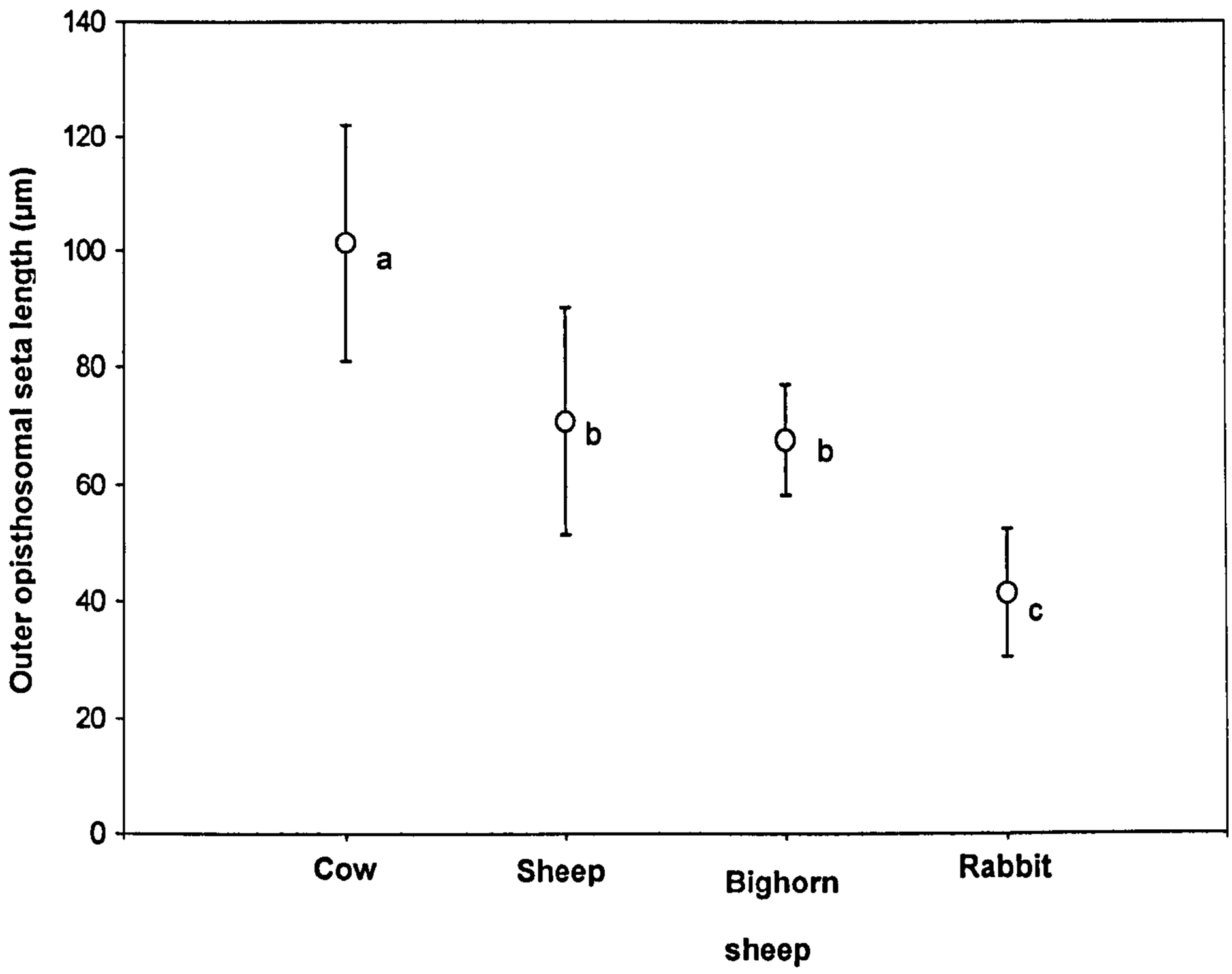
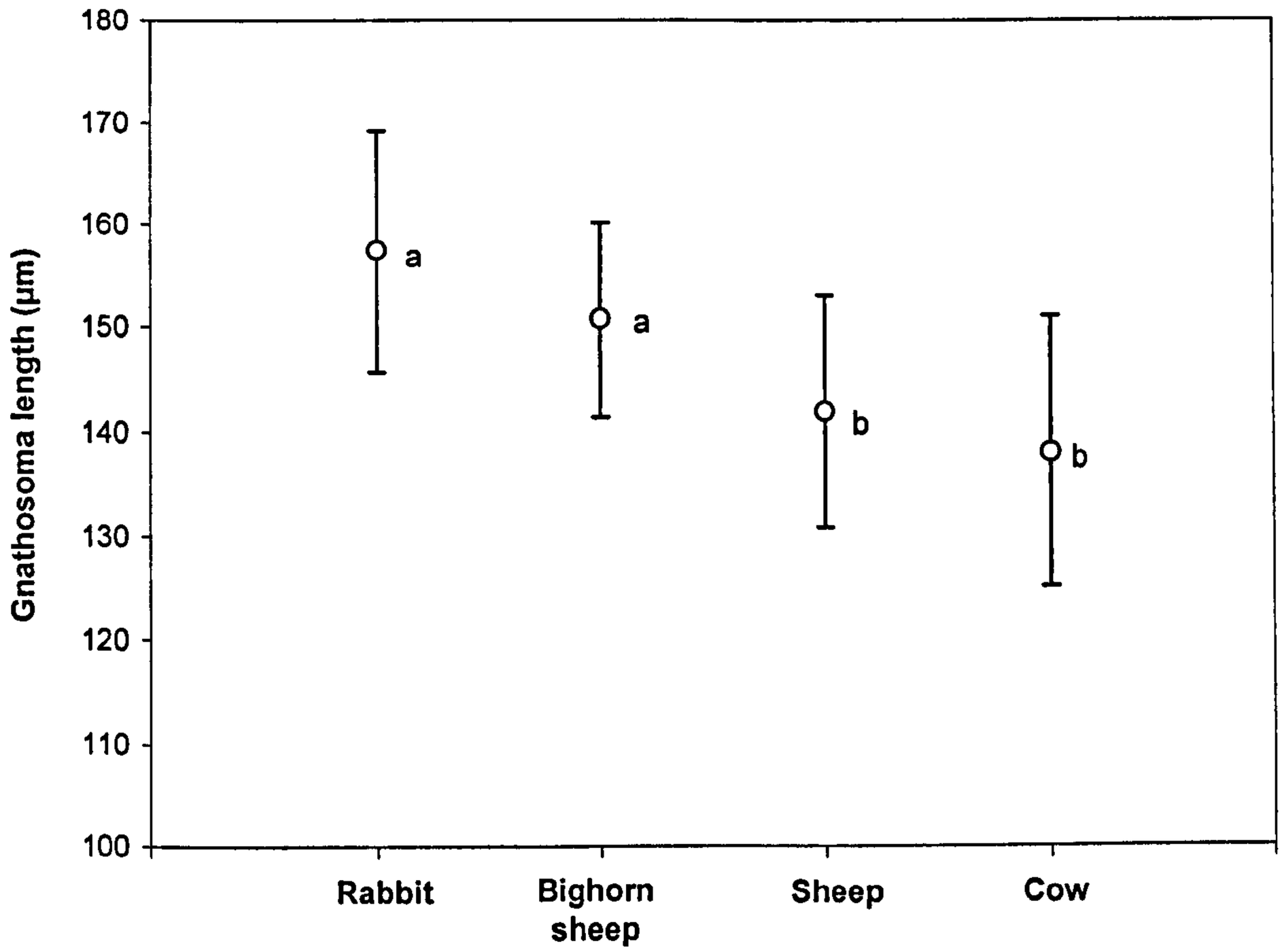


Figure 3.11. Mean gnathosoma length (μm ±s.d.) of adult female *Psoroptes* mites from various host species. Letters indicate groups within which there is no statistically significant difference ($F_{3,208}$=30.42, P<0.001).



Adult females: all samples: pooled isolates

When the discriminant analysis was repeated using mite samples from all host species, excluding goat and elk mites which each have a sample size of just one mite, four significant functions were identified. Function 1 has an eigenvalue of 2.04 and as in the previous analysis, was most highly correlated with outer opisthosomal seta length with a correlation of 0.82 and explains 76.6% of the total variance. Functions 1-3 accounted for 95.0% of the total variance with functions 2 and 3 being most highly correlated with gnathosoma length and leg 1 ambulacrum length, respectively. When functions 1 and 2 are plotted against each other some separation of mites from different host species into groups can be seen though there is considerable variation (Fig. 3.12). In particular, the mites from big horn sheep, white-tailed deer and alpaca are very similar.

When the outer opisthosomal seta lengths were compared for all host species, there was still a significant difference between the seta lengths for different host species ($F_{6,224}$=50.93, P<0.001). However, mite samples from goats and elk were excluded from this analysis as they had a sample size of just 1. Outer opisthosomal setae were again longest in mites from cattle and shortest in mites from rabbits. Post-hoc tests reveal groups within which there is no significant difference between seta lengths. (Fig. 3.13).

Gnathosoma length was found to be the second most important function for discriminating between adult female mites from various host species as was the case when only four mite samples were considered. The longest gnathosoma was found in mites of rabbits and shortest in those of cattle. Gnathosoma length differed significantly between mites of different hosts ($F_{6,224}$=16.51, P<0.001), and post-hoc tests revealed three overlapping groups within which there were no statistically significant differences (Fig. 3.14).

Figure 3.12. Two linear functions of six morphological characters measured on 231 adult female mites as revealed by discriminant analysis. Functions 1 and 2 are most highly correlated with outer opsithosomal seta length and gnathosoma length respectively.

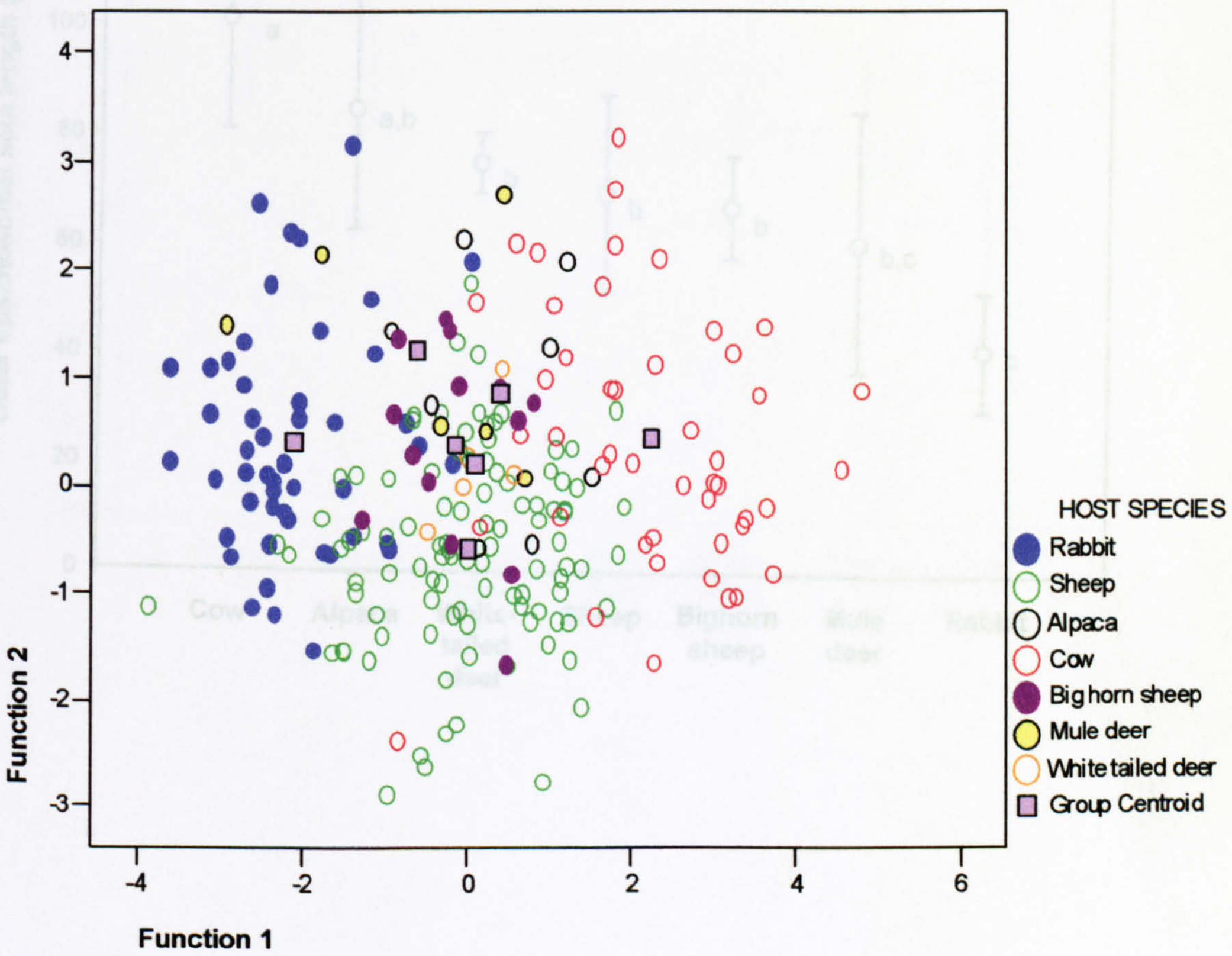


Figure 3.13. Mean outer opisthosomal seta length (μm ±s.d.) of adult female mites collected from a range of host species. Letters indicate groups between which there is no statistically significant difference ($F_{6,224}$=50.93, P<0.001).

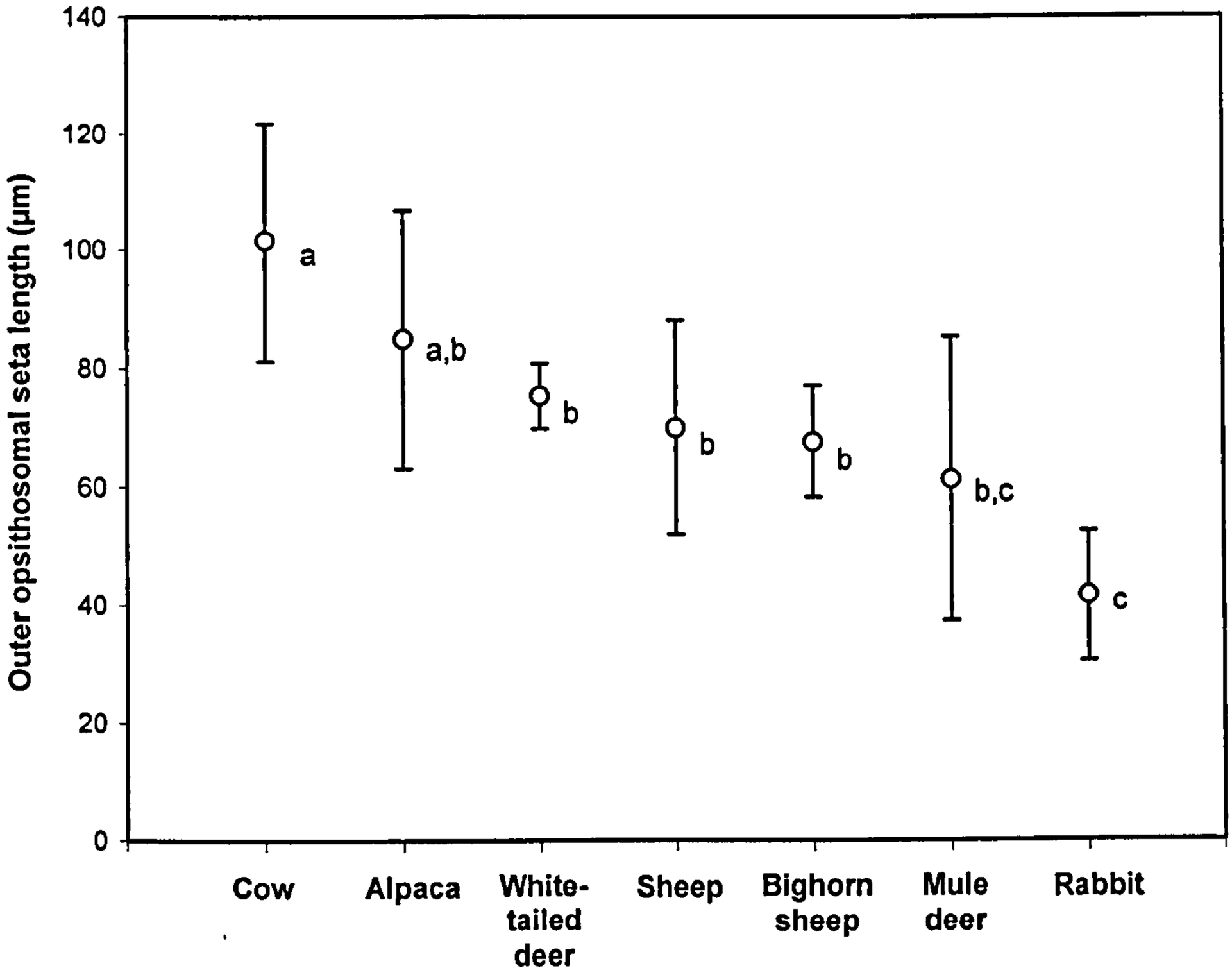
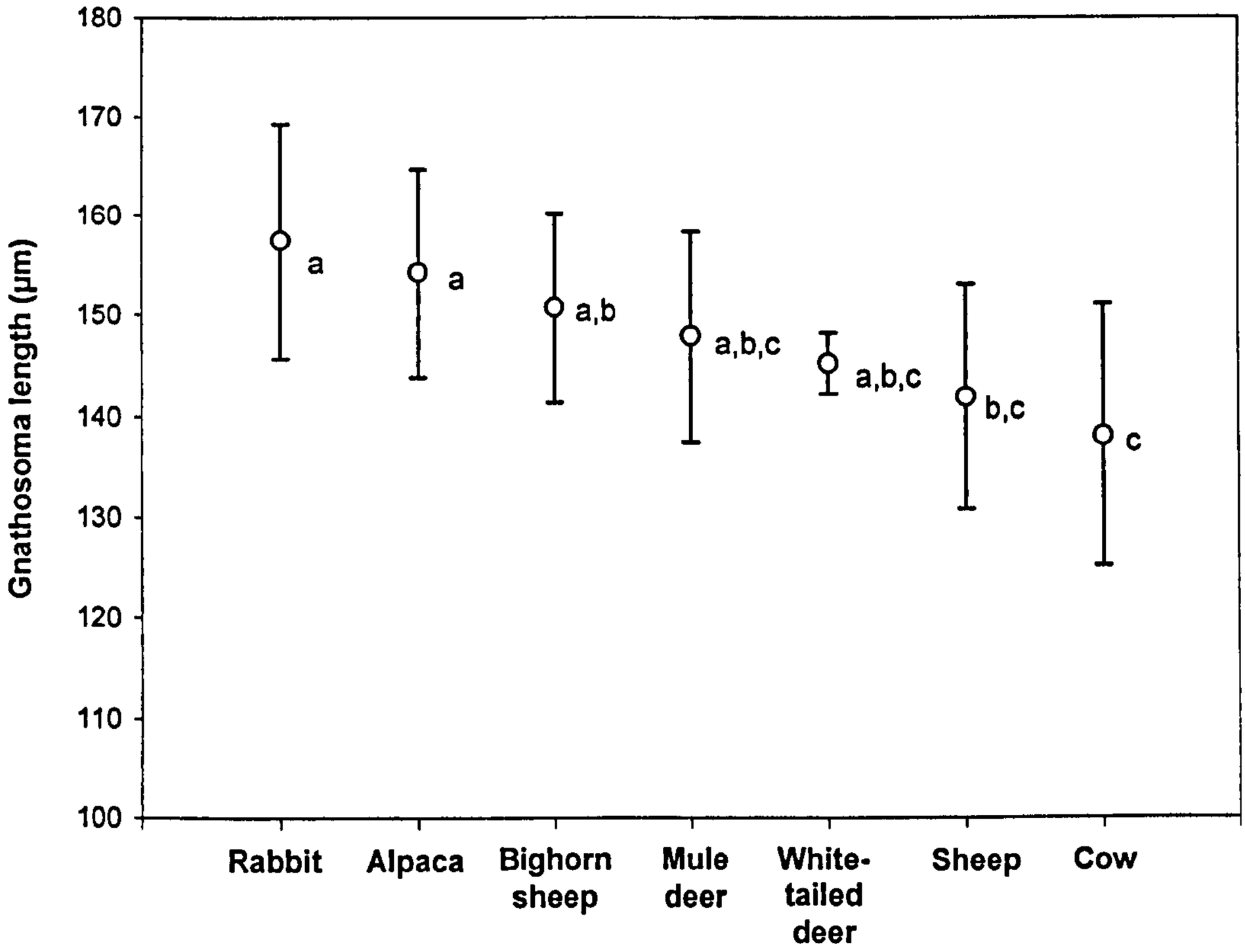


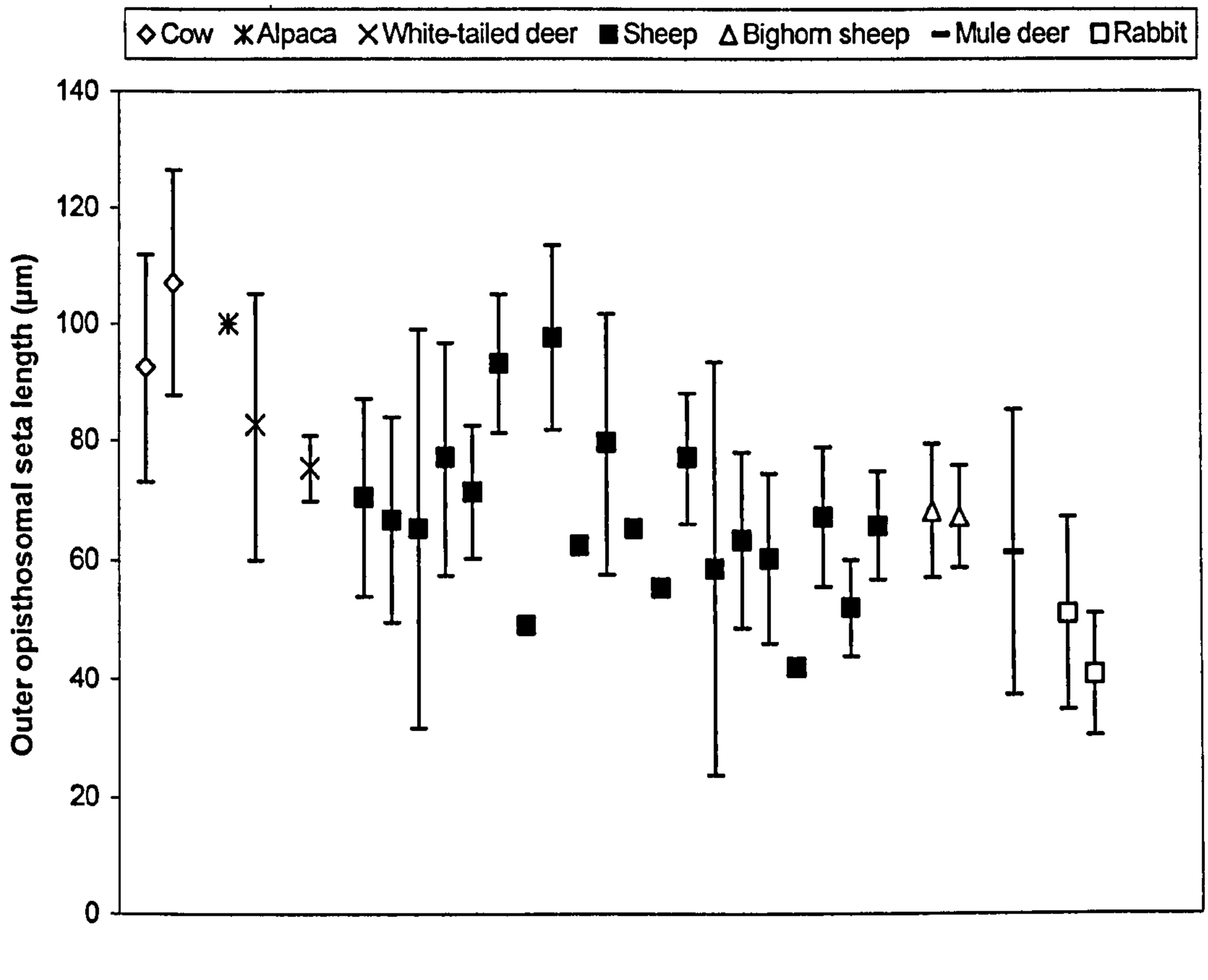
Figure 3.14. Mean gnathosoma length (μm ±s.d.) of adult female *Psoroptes* mites from various host species. Letters indicate groups between which there is no statistically significant difference ($F_{6,224}$=16.51, P<0.001).



Adult females: all samples: all isolates

When the outer opisthosomal seta lengths are considered for individual mite isolates, it can be seen that there is a large amount of variation between mites of the same host species (Fig. 3.15). As was the case for the male mites, there was a significant difference in seta length between the two cattle isolates from Belgium and the USA (t_{45}=-2.502, P=0.016). There was a significant difference between outer opisthosomal seta length from different sheep isolates ($F_{19,82}$=2.08, P=0.012). However when this analysis was repeated using only mite samples where n>1 the significance is greater ($F_{14,82}$=2.41, P=0.007). Post-hoc tests showed that significant differences occured between mites from a South West England flock, and a Yorkshire and Cornwall flock. There were no significant differences between isolates from all other host species.

Figure 3.15. Mean outer opisthosomal seta length (µm ±s.d.) of adult female *Psoroptes* mites of all isolates examined.



3.4 Discussion

The results of this study show that adult male *Psoroptes* mites from different host species can be distinguished between, to some degree, by their morphological characters, with outer opisthosomal seta length being the most important character, as suggested by Sweatman (1958). It has also been shown that outer opisthosomal seta length can distinguish between adult female mites from different hosts, with seta length explaining almost as much variation between the female mite samples as in the male mite samples. However, when mites are examined from a wide range of host species, considerable variation in morphological character measurements becomes apparent. Clearly differences are not host-specific, nor are geographic or body site differences apparent.

In this study, mites from elk and cattle, which would be identified as *P. cervinus* and *P. ovis* respectively using Sweatman's key, here appear almost identical in terms of outer opisthosomal seta length. Outer opisthosomal seta lengths of body mites from cattle and sheep were found to be significantly different from each other in both male and female mites although according to Sweatman's key they should both be described as *P. ovis.* Mites from alpaca, whose actual taxonomic status is still unknown (Strong and Halliday, 1992), were found to be most similar to mites from bighorn sheep in male mites and mites of bighorn sheep and white-tailed deer in the female mites.

Boyce *et al.* (1990) suggest that although outer opisthosomal seta length may be of little use in the identification of possible species, if a fixed character, it may provide information on phylogenetic relationships. If this is the case, it may be that the relatively recently discovered mites of alpacas (Chavez and Guerrero, 1965; D'Alterio *et al.*, 2001) originally came from mite populations of bighorn sheep or white-tailed deer. Alternatively, it may be that the character measurements recorded here are not fixed, but are phenotypically plastic, representing the conditions under

which mites have developed. Such host-induced morphology has been observed in other parasitic mites. Two species of mites of the subgenus *Unionicolides*, *Unionicola U. poundsi* and *Unionicola U. lasellai*, which were originally identified as a single species, are found on different freshwater mussel hosts and adult mites are separated by the shape of particular tarsal claws and setae. Host transfer experiments showed that these morphological characters are dependent on the host species on which metamorphosis of the mites from nymphs to adult occurs and that they are in fact a single species (Downes, 1990). Similarly, more than 30 species were described of mites of the genus *Sarcoptes*. These putative species were based on variable morphological characters which were related to the host species on which the mites were found. However, it is now widely accepted that there is in fact only one, although phenotypically variable, true *Sarcoptes* species (Fain, 1994).

Here, significant differences were found between mites from the same host species but this was not necessarily a result of differing geographical locations. Differences were seen in male and female mites isolated from cattle from the USA and Belgium, and also in male mites from two rabbit samples, both from Bristol, UK. Mites from sheep from all over the UK and Ireland were found not to differ from each other in the male mite samples but differences were found in the female mites although this did not appear to correspond to host location.

A potential problem with a study of this nature is the extent to which fixation and mounting media alter the morphological characters of the mites. Reese *et al.* (1996) examined effects of fixation on thirteen morphological characters on *Psoroptes* mites. They found that fixation in alcohol resulted in significant reductions in body size measurements with measurements decreasing with increasing time of fixation, but the concentration of alcohol had no effect on measurements. Mounting in Hoyer's medium had the opposite effect on body size. Changes in measurements as a result of fixation were most apparent in characters that had the

most internal soft tissue such as body size measurements, however in characters including outer opisthosomal seta length, there was no effect of fixation. Reese *et al.* (1996) recommended consistent methodology in morphometric studies, and this advice was followed in this study.

Overall, the evidence presented here and that of previous studies suggests that *Psoroptes* mites from different hosts do differ morphologically, most notably in the length of the outer opisthosomal setae, and that this is the case in both adult male and female mites. However, these differences are slight and cannot be used reliably to assign host species. Differences between characters of mites of different host species may indicate adaptation to the local microenvironment and not speciation.

The data presented here suggest that Sweatman's (1958) putative five species are not sufficiently distinct to support the claim that they should be classified as separate species. However, differences in the shape of the outer opisthosomal setae may provide support for one distinct species of *Psoroptes*. *Psoroptes natalensis*, first described from mites found on cattle, has opisthosomal setae that are flattened and blade-like at the distal end (Hirst, 1922). Samples of cattle mites examined by Bates and Sayers (2002) were found to have spatulate opisthosomal setae so were regarded as distinct from mite samples examined from other host species. Classification as a single *Psoroptes* species has been suggested by Zahler *et al.* (2000) who proposed naming the species *Psoroptes equi* on the rules of priority, and by Bates (1999) who suggested a return to the name *Psoroptes communis* (Furstenberg). While agreeing that there are good practical reasons to stabilize the nomenclature, according to the law of priority, the senior synonym should take precedence over the junior. Hence, the name *Psoroptes ovis* (Herring 1838) should be applied to synonomise the host-related species described by Sweatman (1958); although whether this also applies to *P. natalensis*, remains to be clarified. There would appear to be no case in favour of the junior synonym, *P. communis*, being adopted.

CHAPTER 4

TACTIC RESPONSES OF *PSOROPTES* MITES

4.1 Introduction

Many species of tick and mite display pronounced tactic and kinetic behaviours in response to chemical, temperature and gravitational cues, which can allow them to make rapid contact with their host or food source (Lees, 1948; Herr, 1991; Carroll *et al.*, 1995; Vail and Smith, 1998; McMahon and Guerin, 2002). For example, specific chemical cues, including animal urine, breath and gland secretions are used by several tick species to locate their host (Carroll *et al.*, 1995). Tarsal gland secretions and urine of white-tailed deer were found to produce an arrestant response in female *Ixodes scapularis* Say and the ticks became stationary on portions of glass tubing coated with the substances (Carroll *et al.*, 1995). Similarly, the ixodid ticks *Amblyomma variegatum* (Fabricius), *Rhipicephalus sanguineus* Latreille, *Ixodes ricinus* Linnaeus and the argasid *Ornithodorous moubata* (Murray) all show attraction towards diluted human breath when placed in an air stream (McMahon and Guerin, 2002). Attraction towards three of the individual components of human breath; acetone, nitrogen oxide and carbon dioxide has also been demonstrated, although a reduced speed of locomotion towards the source of the chemical was observed in the case of acetone and nitrogen oxide. The response shown to a particular substance may vary depending upon the life-cycle stage; *Amblyomma hebraeum* (Koch) shows enhanced responsiveness to carbon dioxide during the host-seeking periods of the life-cycle, with the amount of questing increasing over the six week period following moulting (Anderson *et al.*, 1998). In addition to kairomones,

temperature, light intensity and visual cues are also known to be used by mites and ticks. Responses to heat and vibration by the northern fowl mite, *Ornithonyssus sylviarum* (Canestrini and Fanzago) has been shown to be of importance in host orientation (Owen and Mullens, 2004) and light cues have shown to be of importance to the Banks grass mite, *Oligonychus pratensis* (Banks) in locating itself on its host plant (Li and Margolies, 1991).

Transmission of *P. ovis* mites may occur directly between infested and uninfested hosts (Wilson *et al.*, 1977; Berriatua *et al.*, 1999, 2001). The probability of transmission from infested to naïve hosts has been shown to increase over the period following initial infestation in line with changes in mite abundance, but not to be related significantly to the rubbing activity of infested animals (Berriatua *et al.*, 1999). Transmission may also occur via the environment, with most estimates suggesting an off-host survival time and a period over which infestation remains possible from a contaminated environment, of 15-20 days (O'Brien *et al.*, 1994a; Smith *et al.*, 1999; Meintjies *et al.*, 2002c) with off-host survival being negatively correlated with temperature (Liebisch *et al.*, 1985; Smith *et al.*, 1999, Meintjies *et al.*, 2002c), Whether changes in the behaviour of the mites, such as dispersal activity, might contribute to increasing the probability of transmission, either from infested hosts or via the environment, is currently unknown. Detailed studies of this aspect of mite behaviour, however, may allow a more comprehensive understanding of the process of infection and, furthermore, may contribute towards the successful maintenance of mites *in vitro*.

The aim of the work described here, therefore, was to examine the responses *P. ovis* mites *in vitro* to constant temperatures, temperature gradients and illumination.

4.2 Constant temperature

4.2.1 Materials and Methods

Mites

Adult female mites were removed from the scabs obtained from the ears of infested rabbits as required. Only adult female mites were used because this is the most long-lived life-cycle stage and, as the reproductive stage, is the most critical in terms of population dynamics. Each mite was used only once and mites were not used after they had been off the host for more than 48 h. Between trials, mites were kept in a refrigerator at approximately 4°C. Before each trial, scabs and mites were removed from the refrigerator and maintained at 25°C for approximately 30 to 60 minutes, allowing them to adjust room temperature gradually, before use.

Apparatus and experimental design

The movements of mites were observed in a circular chamber in a glass block (Fig. 4.1). The glass block was placed on a piece of dry filter paper and contained in a petri dish. The chamber consisted of a 10mm hole drilled in the centre of a 25x75mm block of glass, 6mm in thickness. The glass block, filter paper and petri dish were placed on a hotplate (MC60 Temperature controller and warm stage, Linkam Scientific Instruments Ltd, Surrey, UK) and the mites were recorded using a video camera (JVC TK-1281 Colour Video Camera, JVC Professional Products (UK) Ltd, London, UK) which viewed the chamber through a binocular microscope (Leica MZ12, Leica Microscopy and Scientific Instruments Group, Heerbrugg, Switzerland).

Mites were placed in the chamber one at a time and their movements recorded onto video tape for two minutes. The hotplate was pre-warmed to one of five temperatures (30, 35, 40, 45 and 50°C) with a total of 20 mites being assigned randomly to each temperature. Room temperature and humidity were measured at the

start of each trial and were found to be 24.76±0.24 °C and 58.27±0.82 % r.h. respectively.

Analysis

Video recordings of the movements of the mites were displayed on a computer screen and their positions digitised as X-Y co-ordinates and recorded every 5 seconds using a computer programme (written by Dr C. Green in Fortran). This allowed calculation of linear velocity, angular velocity and sinuosity. Linear velocity is defined as the distance moved per unit time, angular velocity as the angle turned per unit time and sinuosity as the angle moved per unit distance moved. Linear velocity, angular velocity and sinusosity were tested for normality using a 1-sample K-S test and then regressed against hotplate temperature (SPSS 12.0, SPSS Inc. Chicago, USA).

4.2.2 Results

Linear velocity had a significant linear relationship with temperature ($F_{1,98}$=40.23, P<0.001, r^2=29.1%); mites moved at greater velocity when placed at higher temperatures (Fig. 4.2). Angular velocity also increased significantly with temperature ($F_{1,98}$=98.09, P<0.001, r^2=50.0%); (Fig. 4.3). Sinuosity did not have any significant relationship with temperature.

Figure 4.1. Glass chamber used to contain mites for observation with a video camera at different temperatures.

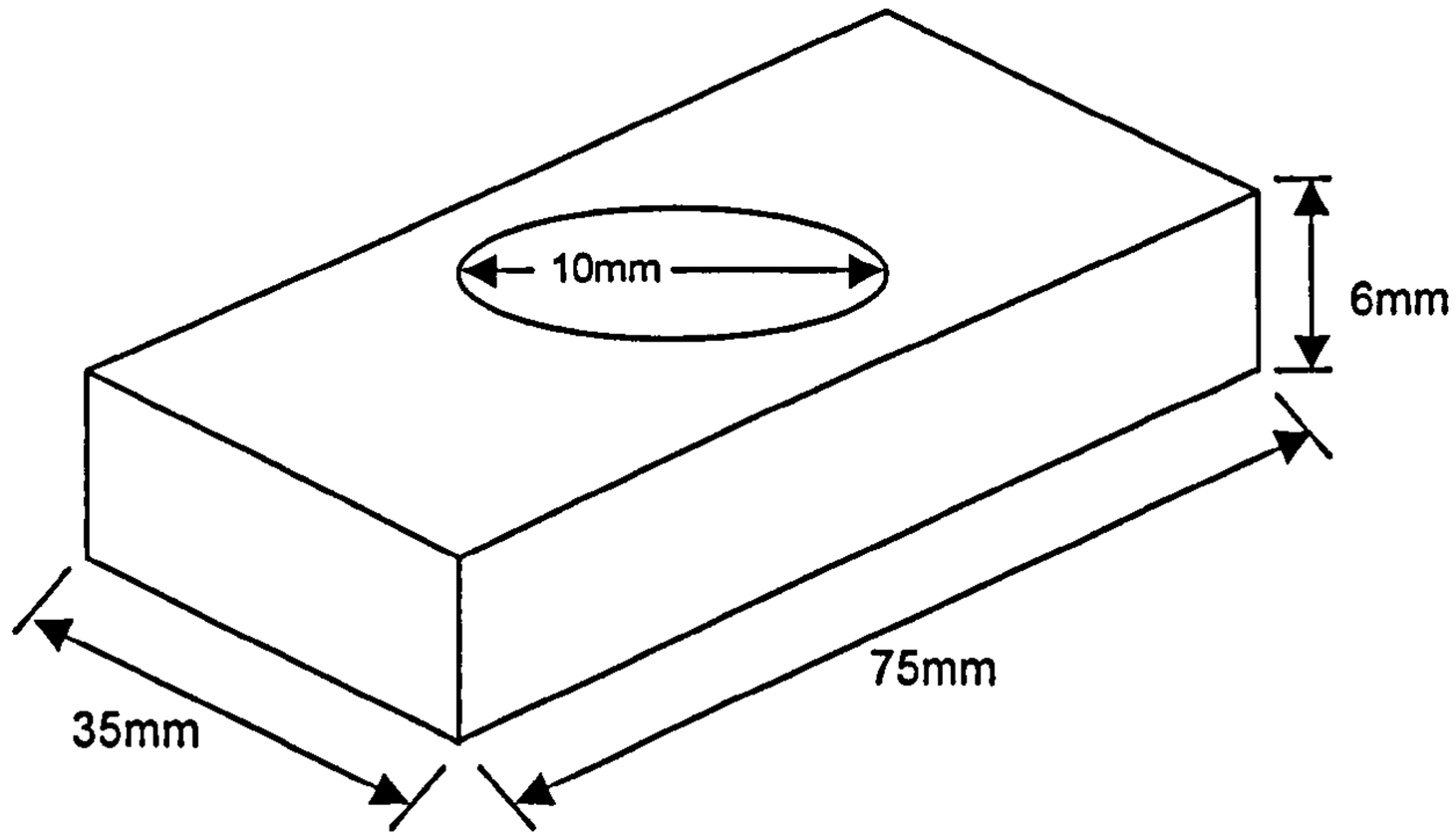


Figure 4.2. Linear velocity of *Psoroptes ovis* mites when placed on a hotplate of constant temperature (Fitted line: $y=2.1x10^{-5}x-3.3x10^{-4}$)

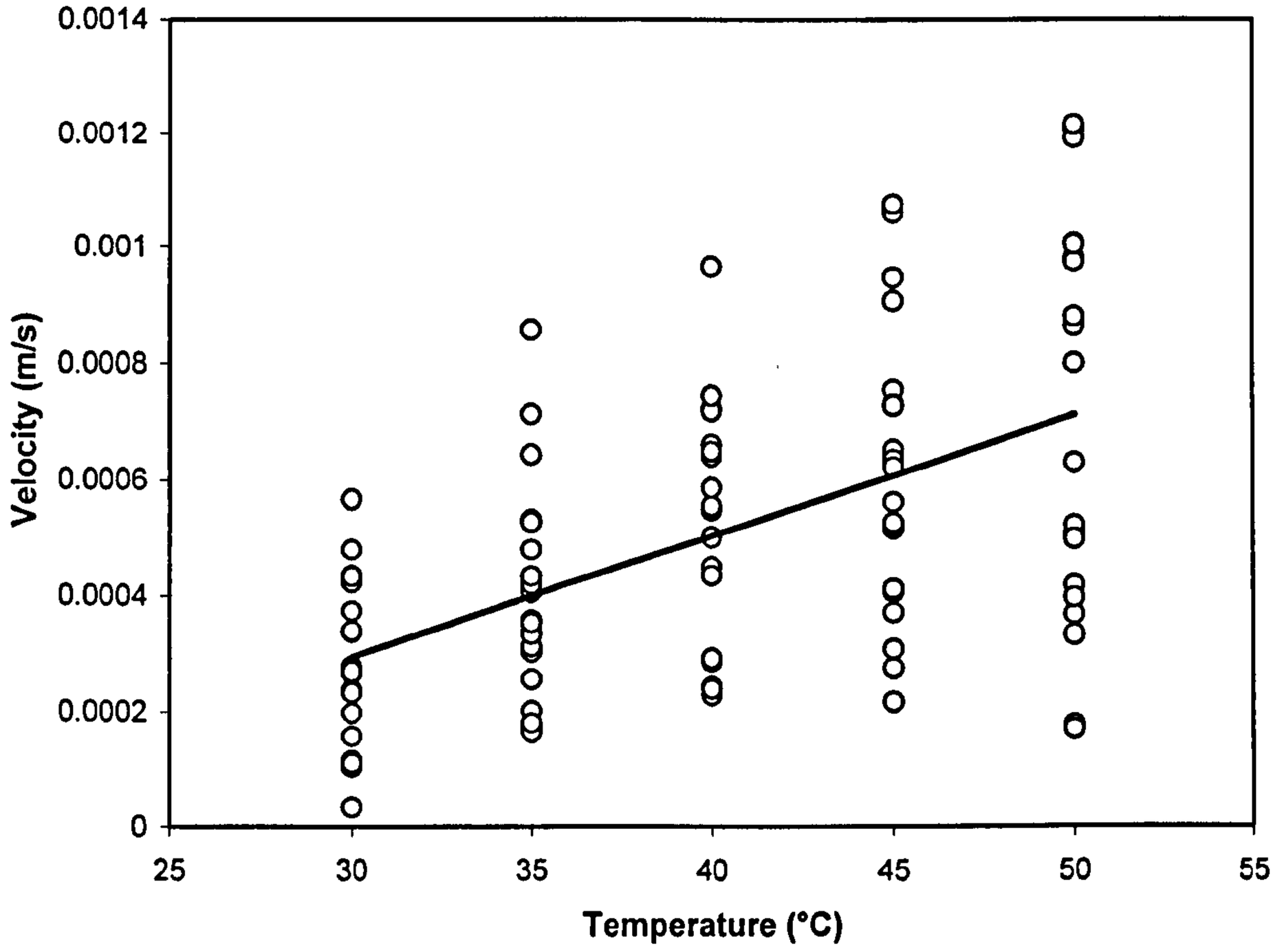
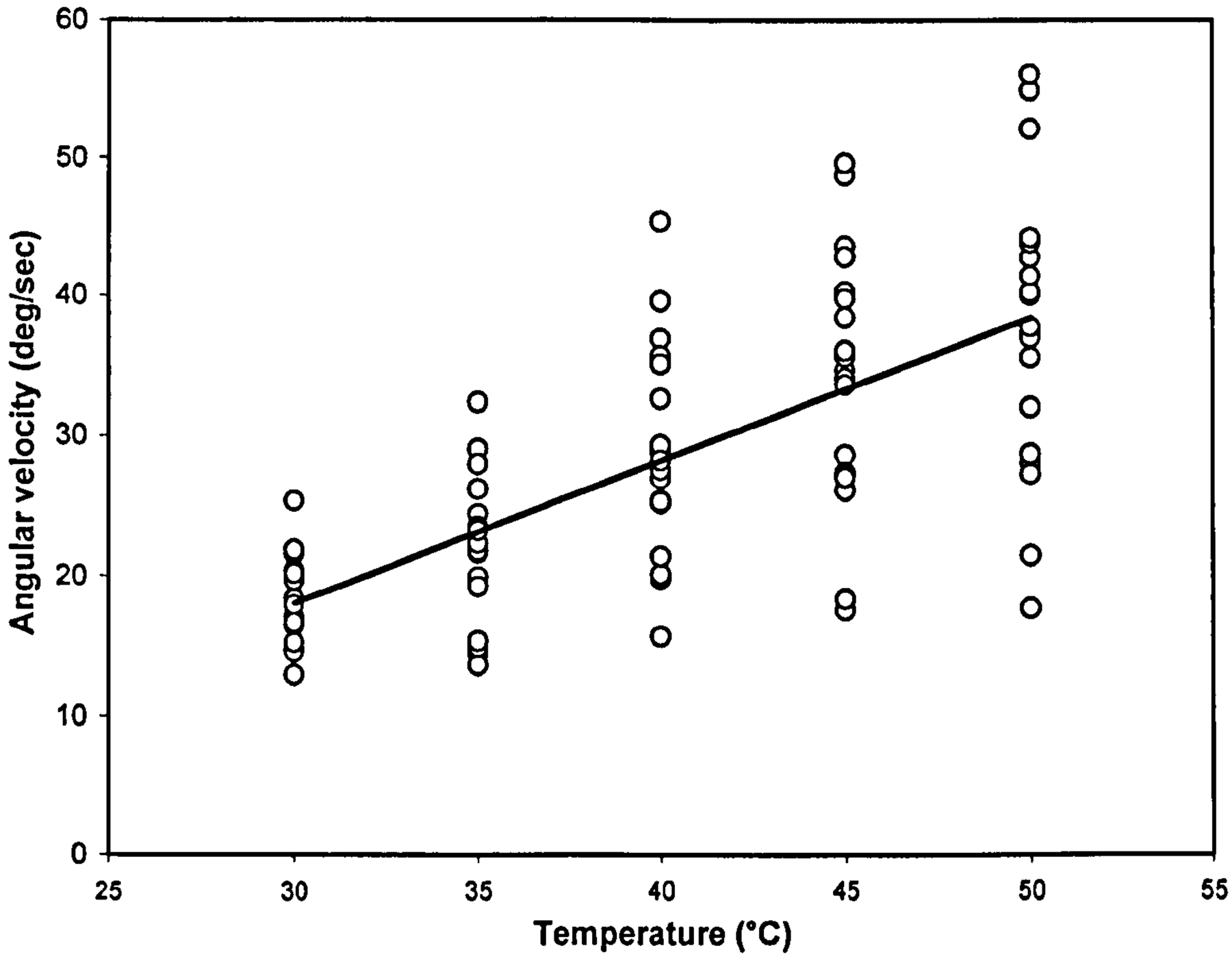


Figure 4.3. Angular velocity of *Psoroptes ovis* mites when placed on a hotplate of constant temperature (Fitted line: y=1.01x-12.3, r^2=50%)



4.3 Temperature and light gradients

4.3.1 Materials and Methods

Mites

Adult female mites only were used for these experiments.

Apparatus and experimental design

To monitor the behaviour of the mites, an arena was constructed to provide a surface for the mites to walk over (Fig. 4.4). For this, a sheet of aluminium (450x165x1.2mm) was bent so that it presented a flat vertical surface (240x165mm) with two (105x165mm) projections at its top and bottom, bent at 90° to the front surface. Two thermostatically controlled hotplates (Dishwarmer 2, 220-250V 170W, Photax, UK) were attached to the top and bottom projections of the aluminium sheet. The hotplates allowed a temperature gradient to be produced across the longitudinal axis of the arena. Two 15W fluorescent lights, 420 mm in length and 25 mm diameter (Duro-lite®, True-lite, Illinois, USA) were also clamped at the top and bottom of the arena surface to allow a light gradient to be produced. A rectangular (240x165mm) piece of white cotton (Arcade Sewing Machine Co. Ltd., Bristol, UK) was attached to the front surface of the arena. A line was drawn horizontally across its centre. Prior to each trial, the sheet of cotton was immersed in water and then excess water wrung out to provide a dampened surface. To standardise and, if necessary, correct for differences in wetness, the cotton was weighed wet at the start of each trial and at the end, the difference calculated.

All the apparatus, arena, hotplates and lights, were then placed inside a larger aluminium outer chamber (Fig. 4.5). The lid of the chamber could be closed to exclude any exterior light. Air was sucked into the chamber and through a filter of activated charcoal by a 10 cm diameter fan, to eliminate any organic sources of

odour that might have affected the behaviour of the mites. A small perspex window in the lid of the chamber allowed a video camera (Swallow, MCD 2075, Custom Cameras Ltd, Wells, UK) to record the movements of the mites from a distance of 60 mm.

Three adult female *P. ovis* mites were used in each trial. The mites were placed approximately 5 cm apart and, at this distance, there was no apparent interaction between individuals. They were removed from the scab and placed on the centre line of the piece of cotton. After closing the lid on the aluminium outer chamber, the video camera was turned on and the movements of the mites were recorded for 5 minutes.

Temperature and light gradients were created across the test arena using the hotplates and fluorescent lights. To produce a temperature gradient, one of the hotplates was turned on at a temperature of between 30-50°C, while the other one was turned off. The gradient was allowed to establish for at least 20 minutes before the experiment commenced. Temperature was measured at five evenly distributed points along the vertical axis of the test arena and was recorded at the start, middle and end of each five minute trial. Room temperature was maintained using a thermostatically controlled fan heater; average room temperature over the course of the trials described here was recorded as 25.9±0.1°C. As a control, neither of the hotplates were turned on so that no temperature gradient was present.

Figure 4.4. Aluminium sheet arena, with hot-plates and fluorescent lights, used to create temperature and light gradients. A video camera and recorder were used to record movement of the mite *Psoroptes ovis*.

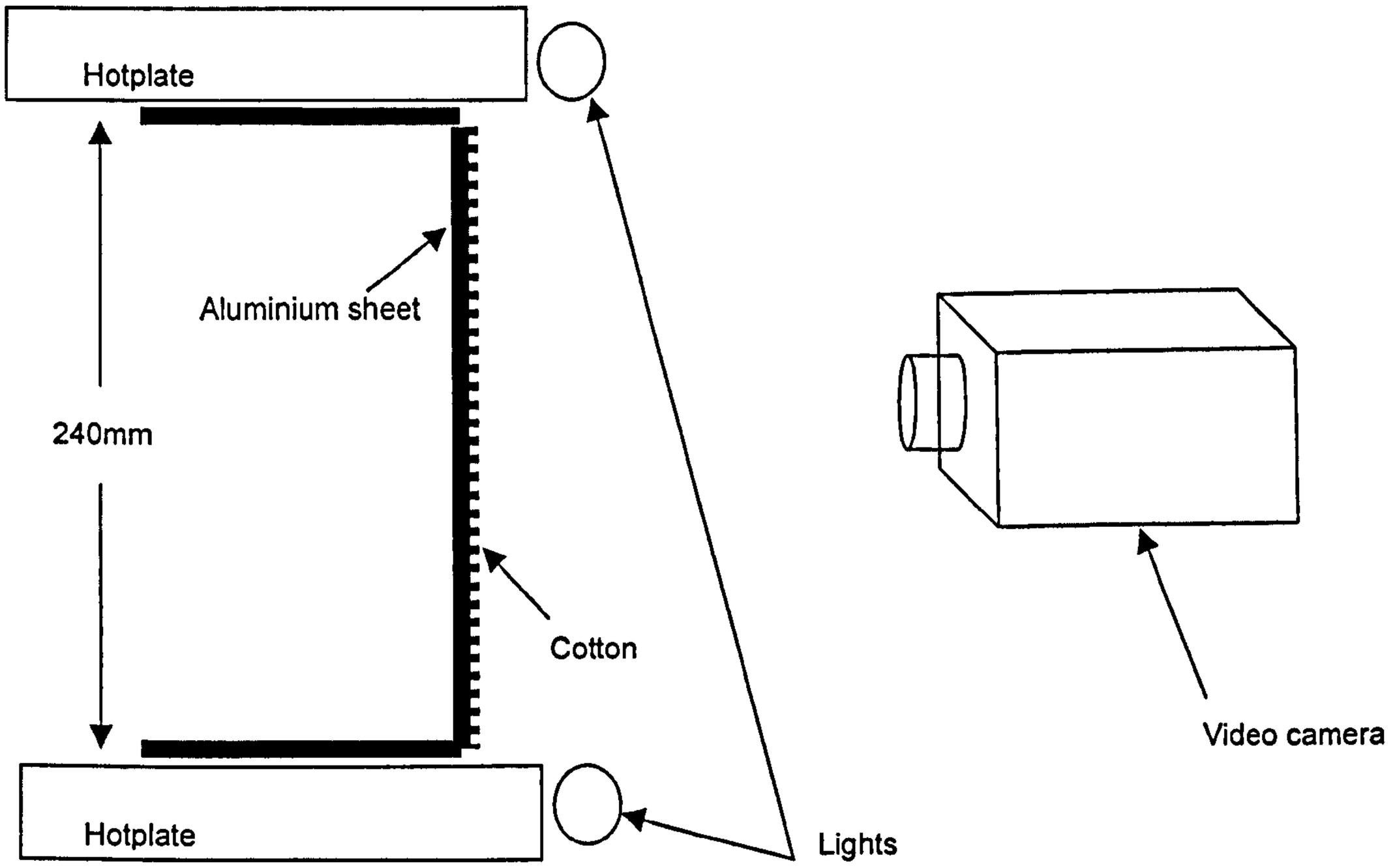
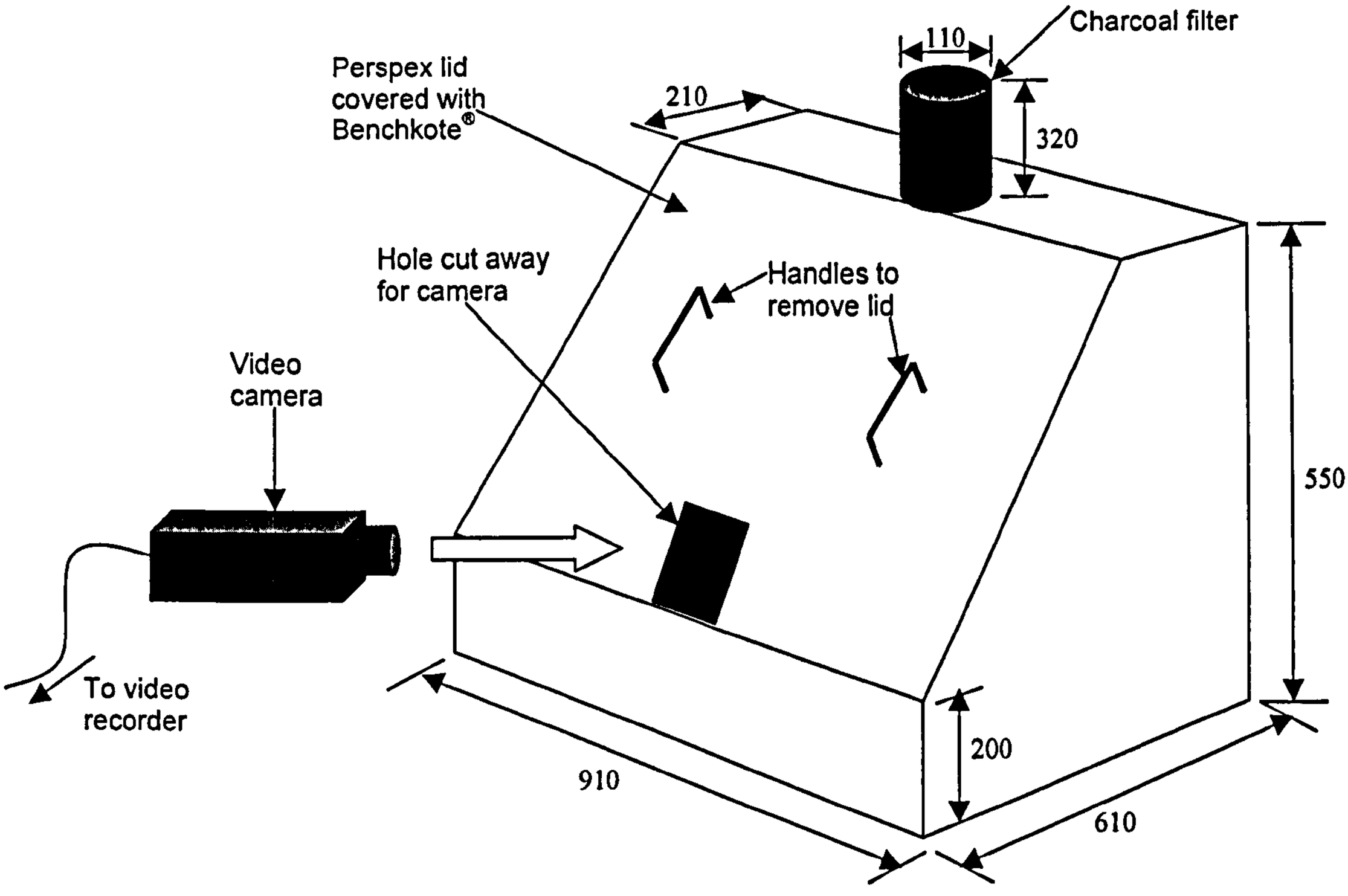


Figure 4.5. Aluminium box used to enclose apparatus to exclude exterior light and eliminate organic sources of odour. (All dimensions are in mm).



Trials were run with either both fluorescent lights turned on or, to produce a light gradient, with only one of the fluorescent lights, either top or bottom, turned on. It was assumed that with only one light turned on, light intensity declined detectably from the light source over the surface of the test arena, although this was not measured. Controls were run with both lights turned off, in which case the video camera was not used; trials were carried out in total darkness and at the end of five minutes the lights were switched on and the position of the mites was marked on the cotton surface.

Each of the 28 light and temperature combinations were run six times, each with three mites. These light and temperature combinations consisted of seven different temperature gradients (both hotplates turned off, or either top or bottom hotplate set at 31.1±0.1, 39.5±0.2, 48.35±0.1°C) and four different light combinations (both lights turned on, both lights turned off or either top or bottom light turned on only). These combinations meant that the effects of temperature and light could be looked at individually or together. Ambient room relative humidity was recorded at the start of each trial and varied little during the experimental work reported here (mean = 47.2%, s.e.±0.5% r.h.).

Analysis

The video recordings of the mite movements were displayed on a computer screen and their positions digitised as X-Y co-ordinates and recorded every 20 seconds using a computer programme (written by Dr C. Green in Fortran). This allowed the calculation of vertical distance travelled, which is the primary variable considered here. Vertical distance travelled is defined as the straight line distance travelled perpendicular to the line of origin. A positive value therefore indicates an upward movement and a negative value indicates a downward movement. Linear

velocity, angular velocity and sinuosity of the mites were also measured. Linear velocity is defined as the distance moved per unit time, angular velocity as the angle turned per unit time and sinuosity as the angle moved per unit distance moved. The measures of vertical distance travelled, linear velocity, angular velocity and sinuosity were tested for normality using a 1-sample K-S test and Levene's test was used to check for homogeneity of variances. Vertical distance travelled was treated either as the dependent variable in analysis of variance where light treatment (on/off) was a factor, or as the dependent variable in linear or polynomial regression, where temperature difference was the independent variable. The measures of velocity, angular velocity and sinuosity were taken as the dependent variable in an ANCOVA where light treatment was used as a fixed factor and temperature at the mid-point of the arena controlled for as a co-variate. In this case directional movement was not of primary importance, hence temperature was measured at the mid-point of the arena instead of the temperature difference across the whole arena. Tukey multiple range tests were used to explore the differences between classification groups following analysis of variance.

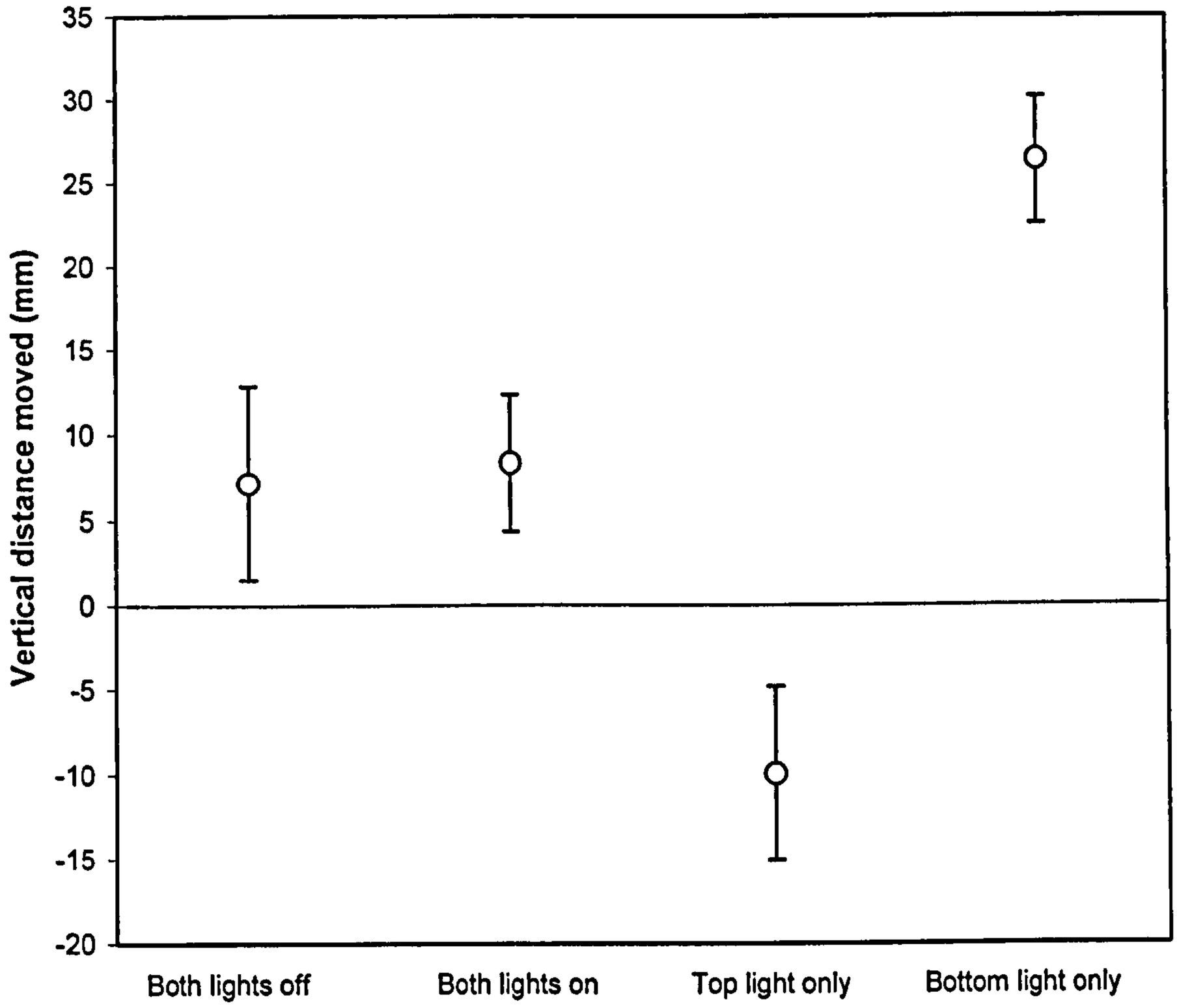
4.3.2 Results

Multiple regression analysis was used first to consider the possibility that environmental factors, which were not controlled in the trials, might have had a significant impact on the vertical distance moved, linear velocity, angular velocity or sinuosity of the mites. For all trials, the vertical distance moved, linear velocity, angular velocity and sinuosity was regressed against the ambient humidity and temperature of the laboratory, the water content of the cotton covering the test arena and the absolute temperature of the hotplate that was not turned on. No consistent significant effect of any of these environmental factors was found and it was

concluded therefore that they had no impact on the results and were not considered further.

The presence and direction of the illumination had a significant effect on the distance and direction moved by the mites ($F_{3,59}$=8.41, P<0.001; Fig. 4.6). With no temperature gradient and both lights on or both off, so that there was no unidirectional light gradient, the mites moved relatively little and there was no significant difference in the distance moved between these two light treatments (Tukey multiple range test, P>0.05). There was, however, a slight tendency to move upwards (t_{33} =2.28, P= 0.03). In contrast, when the illumination was from above only the mites moved down below the line of origin, but when illumination was from below only, the mites moved upwards. The distances moved when the arena was illuminated from above or below only, were significantly different (Tukey multiple range test, P<0.05) and both were significantly different from the distances moved with either both or no lights turned on (Tukey multiple range test, P<0.05). The data show that the mites moved away from the source of the illumination when this was unidirectional (Fig. 4.6).

Figure 4.6. The mean distance moved (±s.e.) by *Psoroptes ovis* mites when subjected to unidirectional or non unidirectional light gradients.



With a temperature gradient and both lights turned on, there was a significant relationship between the vertical distance moved and temperature difference between the top and bottom of the arena (Fig. 4.7; $F_{1,114}$= 55.4, P<0.001, r^2=32.7%). As the gradient became greater and hotter at the top, the mites moved upwards; when the gradient became greater and hotter at the bottom, the mites moved down. Hence, the mites consistently moved towards the area of highest temperature, although, as shown, the variance around the fitted regression line is relatively high (Fig. 4.7). When this trial was repeated with no lights on, a similar significant but highly variable relationship was found (Fig. 4.8) ($F_{1,123}$=14.7, P<0.001, r^2= 10.7%). Neither the slopes nor intercepts of the two regressions differed significantly, suggesting that the mites responded in the same way to the temperature gradient, regardless of whether the lights were on or off.

When illumination was from above only, the movement of the mites in response to the temperature gradient was displaced downwards with respect to the origin (Fig. 4.9a). However, there was still a significant positive polynomial relationship between temperature difference and vertical distance travelled ($F_{1,108}$=30.98, P<0.001, r^2=36.25%), but the mites did not move downwards so far when the gradient was hotter at the top. Similarly, when illumination was from below only, the movement of the mites was displaced upwards (Fig. 4.9b). In this case there was a weak, but significant, polynomial relationship between temperature difference and vertical distance moved ($F_{1,94}$=5.24, P=0.007, r^2=10.1%), but in this case the movement upwards was least when the temperature gradient was smallest.

Figure 4.7. Vertical distance moved by *Psoroptes ovis* mites in the presence of a temperature gradient but in the absence of any unidirectional light gradient (with both lights turned on)(Fitted line: $y=1.3283x + 8.0165$ $r^2=32.7\%$).

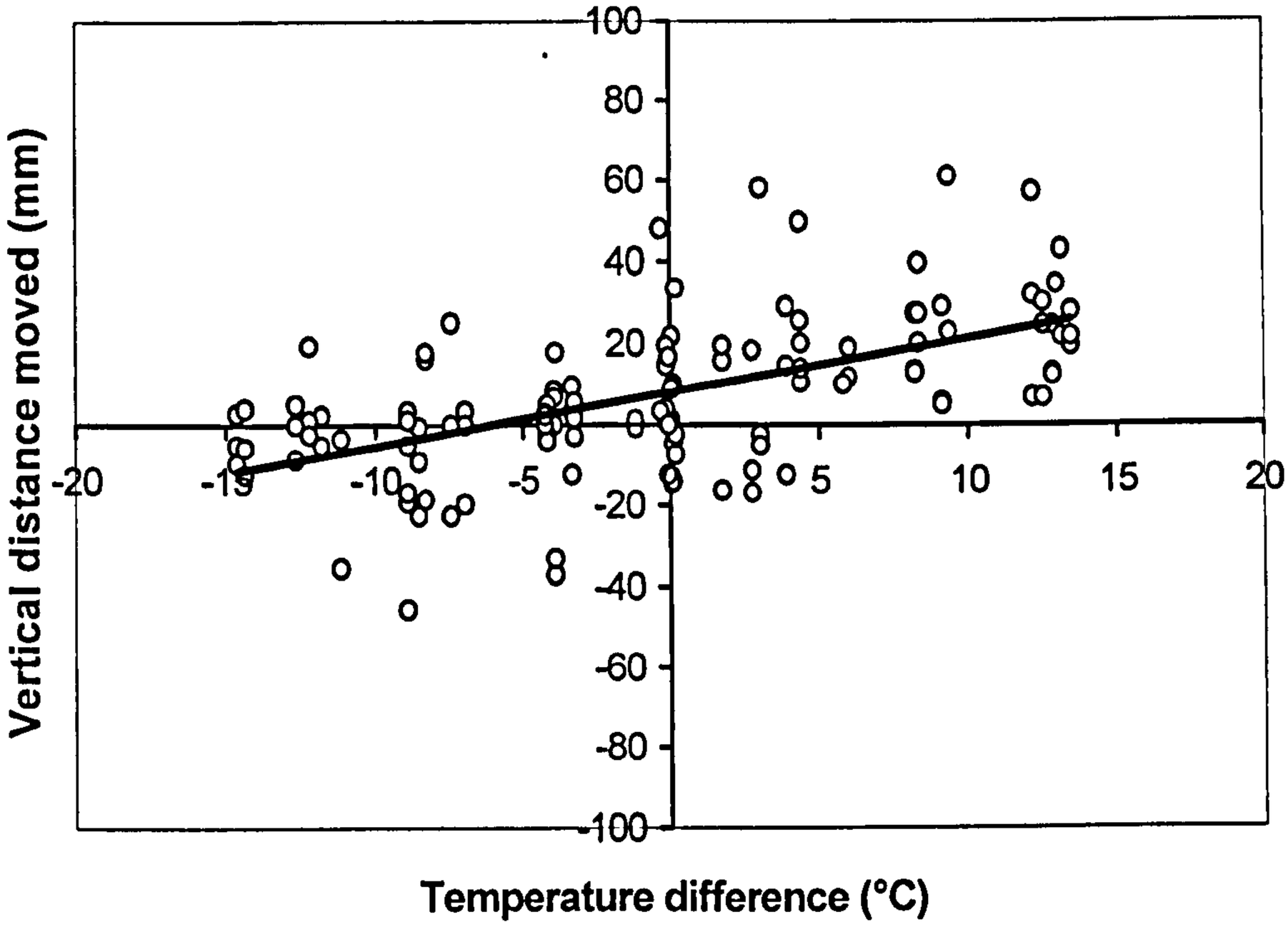


Figure 4.8. Vertical distance moved by *Psoroptes ovis* mites in the presence of a temperature gradient but in the absence of any unidirectional light gradient (with both lights turned off) (Fitted line: $y=0.878x + 5.789$, $r^2=10.7\%$).

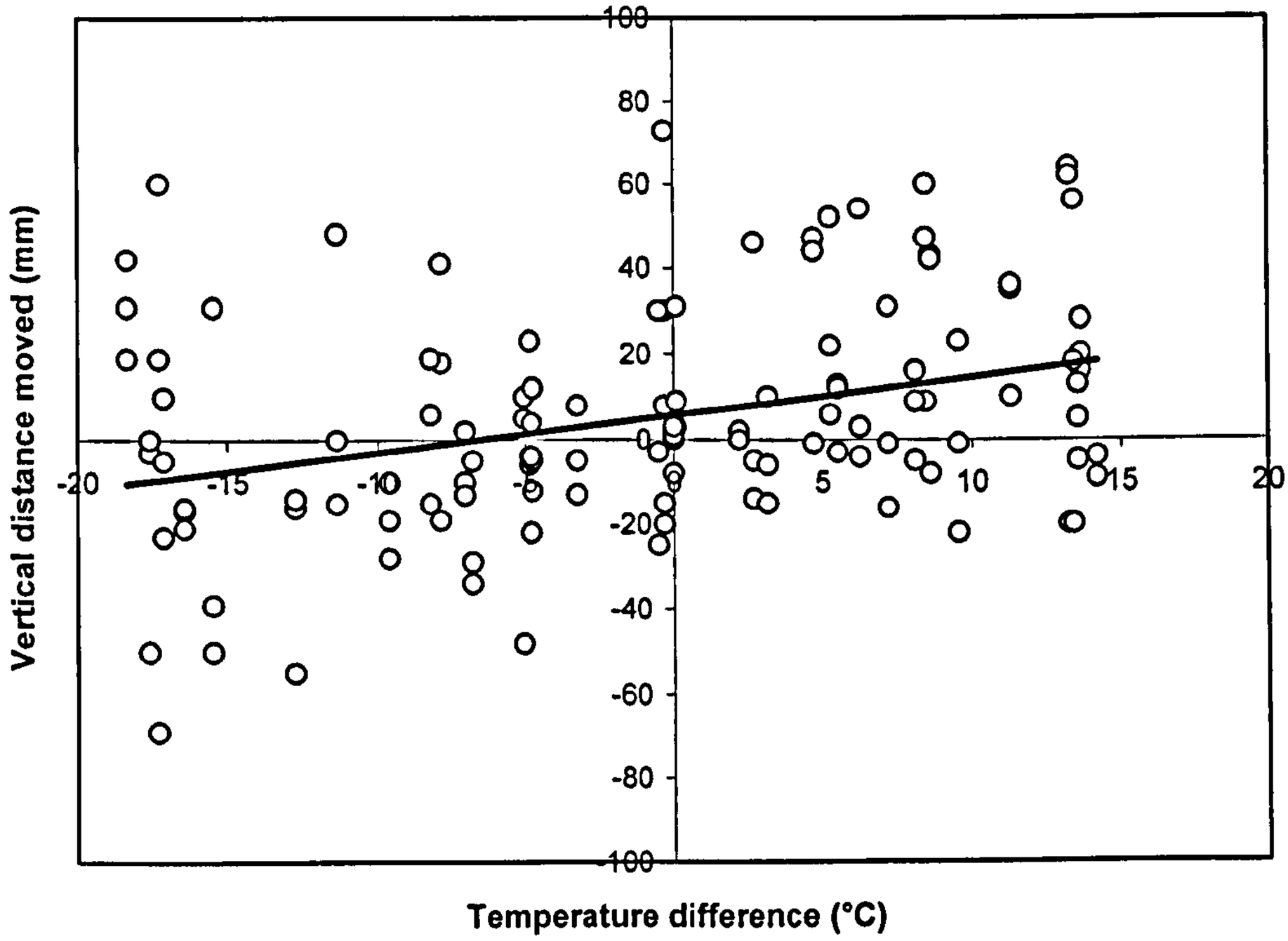
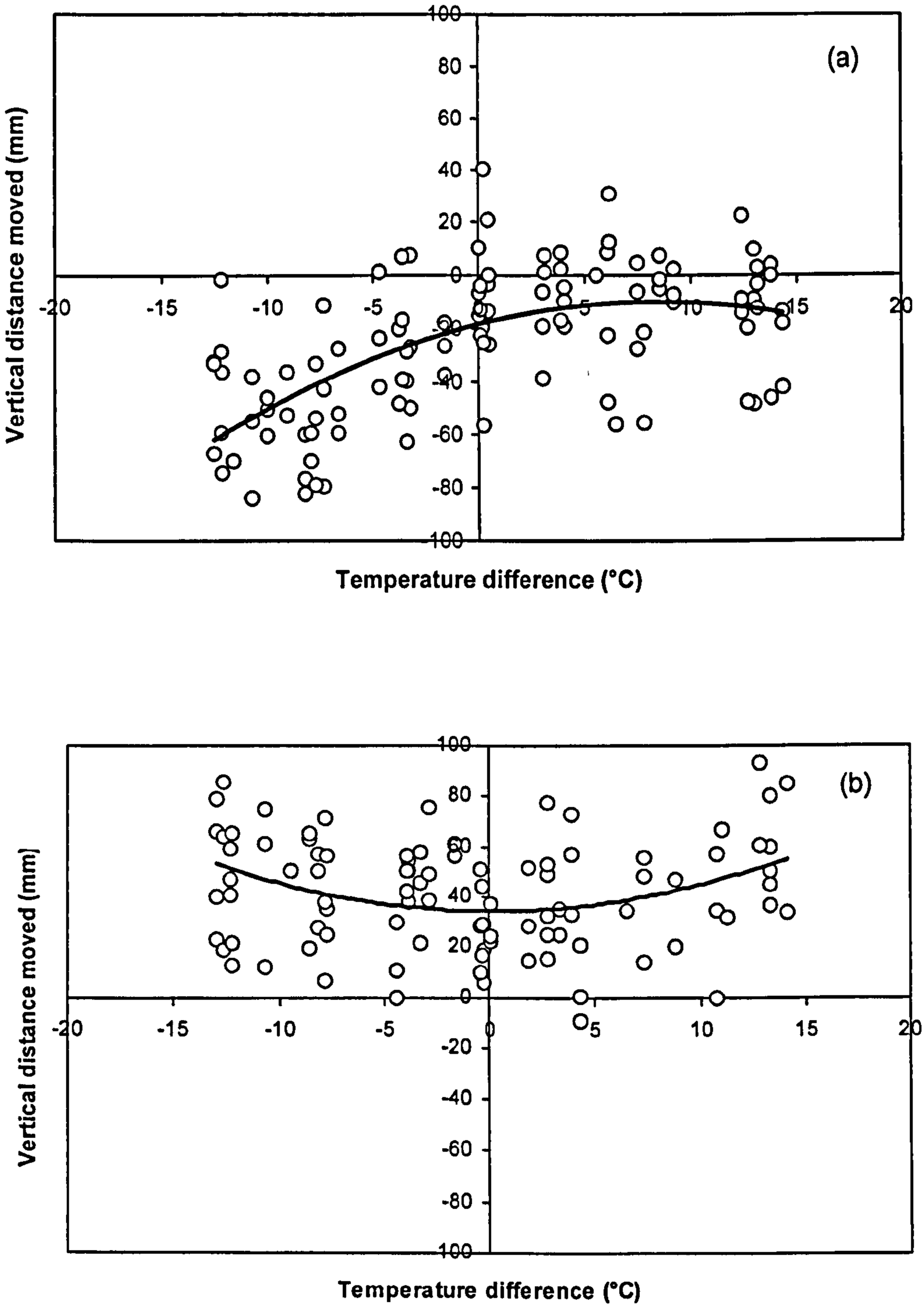


Figure 4.9. Vertical distance moved by *Psoroptes ovis* mites in the presence of a temperature gradient and a unidirectional light gradient from (a) above (fitted line: $y= -0.1185x^2+1.995x-18.256$, $r^2=36.2\%$) and (b) below (fitted line: $y=0.1073x^2-0.0241x+34.282$, $r^2=10.1\%$).



Linear velocity of the mites across the arena did not vary significantly when there was a uni-directional light gradient or no light gradient ($F_{2,317}$=2.97, P=0.053). There was a significant linear relationship between velocity and temperature at the mid-point of the arena, when all light treatments are pooled together ($F_{1,319}$=152.15, P<0.001, r^2=32.3%). When mites were at higher temperatures, their velocity was greater (Fig. 4.10).

Controlling for temperature at the mid-point of the arena, the direction of light had a significant effect on the angular velocity of the mites ($F_{2,317}$=6.52, P=0.002). Angular velocity was significantly greater when mites were placed in a uni-directional light gradient (when light was from above or below) compared to when there was no uni-directional light gradient. The presence of a uni-directional light gradient, whether light was from above or below only, did not significantly affect the rate of turning of the mites. Angular velocity was had a significant linear relationship with temperature, when there was no unidirectional light gradient ($F_{1,111}$=42.93, P<0.001, r^2=27.9%); a uni-directional light gradient with light from above ($F_{1,110}$=10.79, P=0.001, r^2=8.9%) and a uni-directional light gradient with light from below ($F_{1,94}$=8.56, P=0.004, r^2=8.3%). At higher temperatures angular velocity of the mites was increased (Figs. 4.11 and 4.12).

Sinuosity was square root transformed to normalise the data. Sinuosity did not differ significantly between light treatments and showed no significant relationship with mid-point temperature.

Figure 4.10. Linear velocity of *Psoroptes ovis* mites in the presence of a temperature gradient under both unidirectional and bidirectional light gradients (fitted line: $y=1.1x10^{-5}x - 8.7x10^{-5}$, $r^2=32\%$).

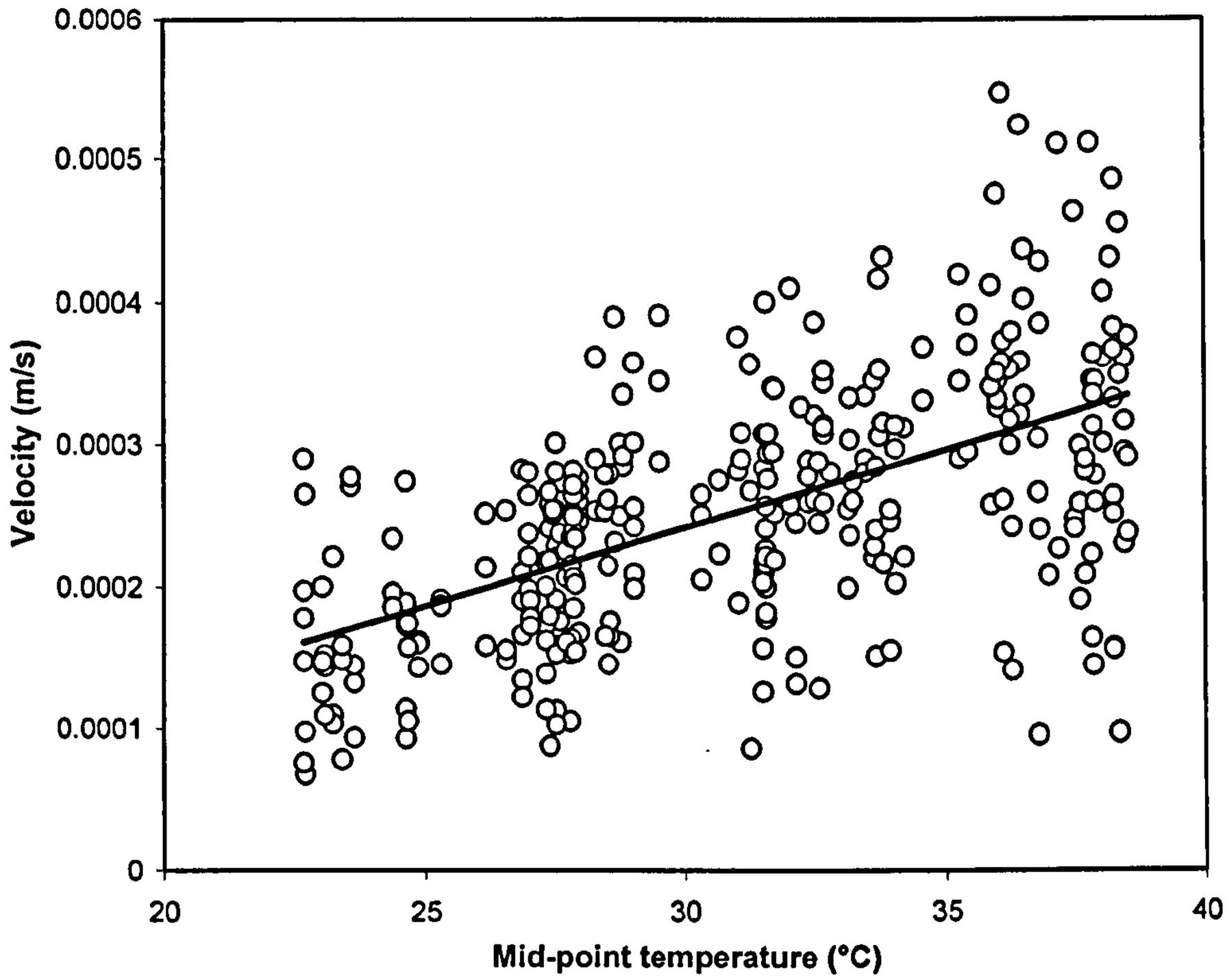


Figure 4.11. Angular velocity of *Psoroptes ovis* mites in the presence of a temperature gradient and in the absence of any uni-directional light gradient (with both lights turned on). (Fitted line: y=0.1243x-0.5908, r^2=27.9%).

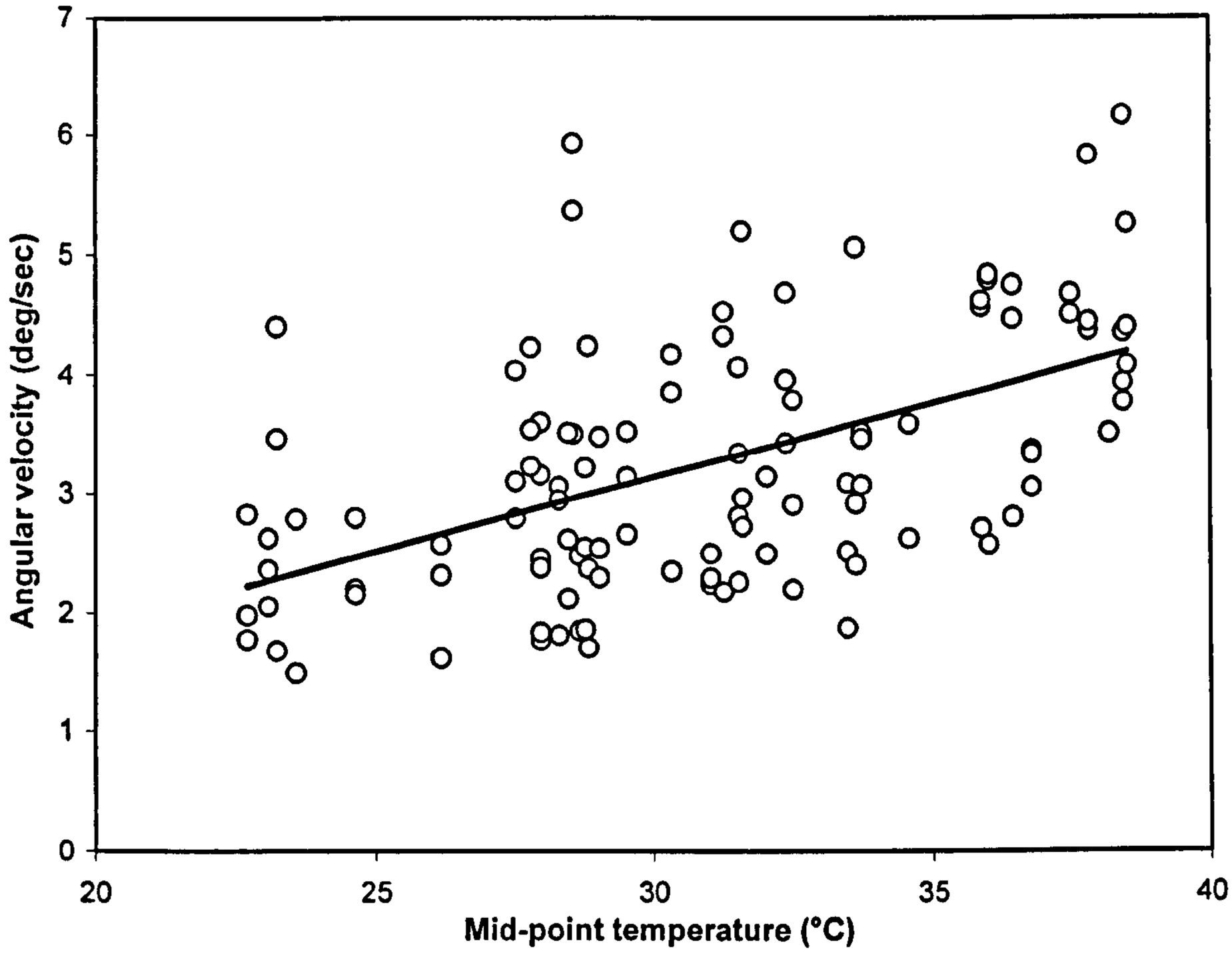
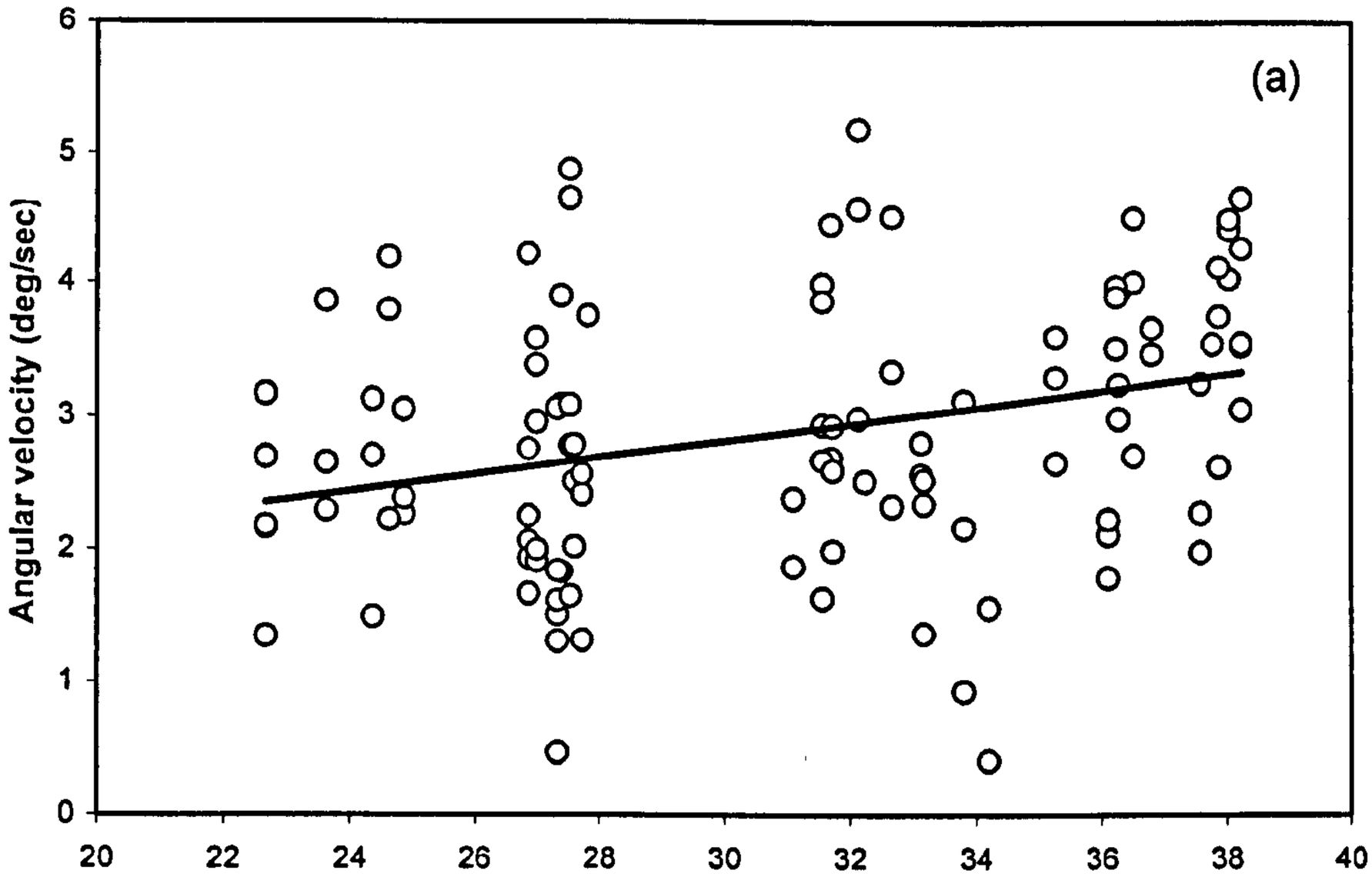
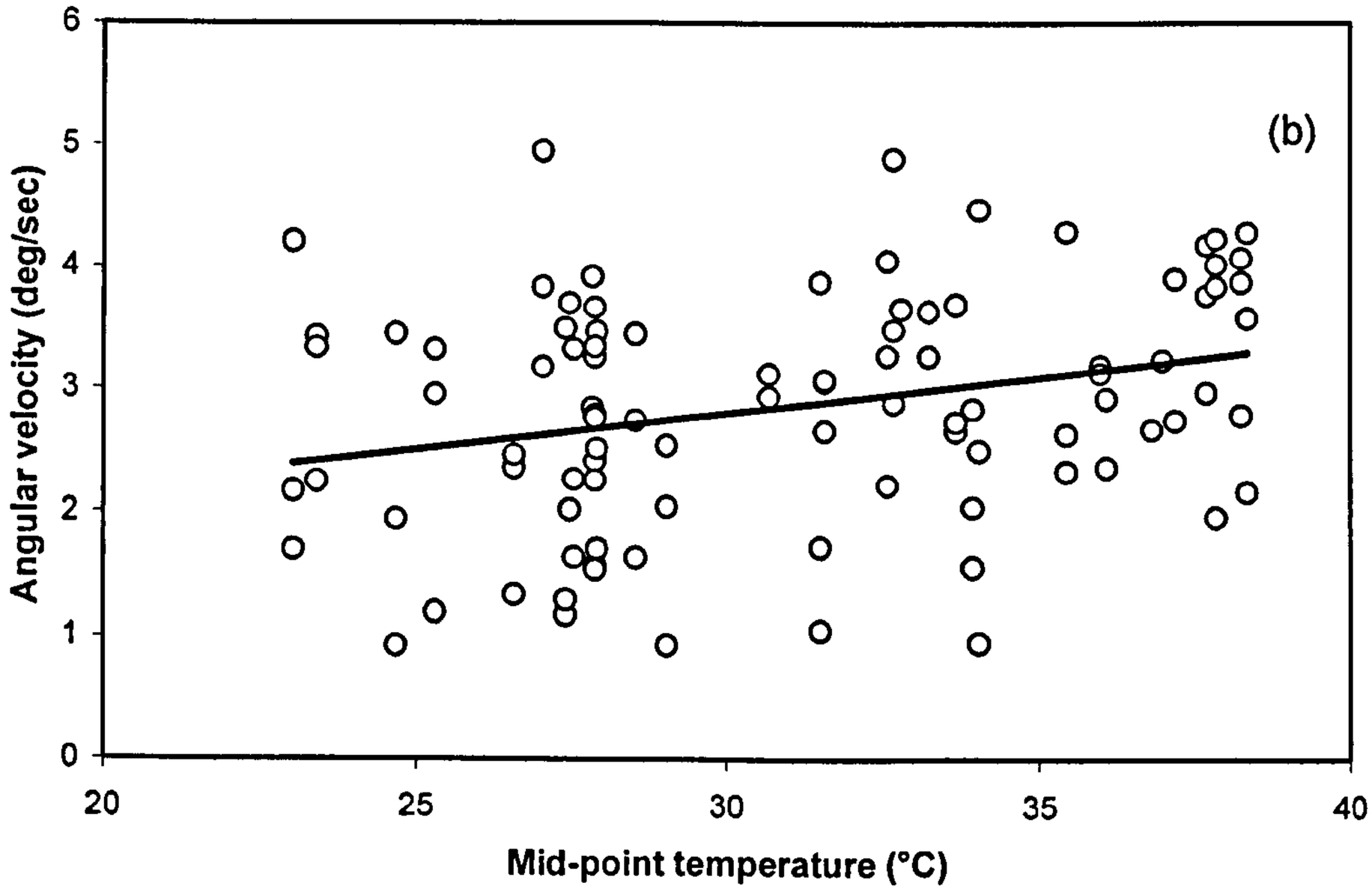


Figure 4.12. Angular velocity of *Psoroptes ovis* mites in the presence of a temperature gradient and a unidirectional light gradient from (a) above (fitted line: y=0.0619x+0.9561, r^2=8.9%) and (b) below (fitted line: y=0.0594x+1.0152, r^2=8.3%).





4.4 Discussion

The results of the present study showed that adult female *P. ovis* mites responded positively to temperature and negatively to gravity and light and showed increased linear and angular velocity when exposed to higher temperatures. Mites moved towards the hotter end of a temperature gradient, regardless of whether this was above or below. Similar responses are well known in ticks. For example, *I. ricinus* larvae and nymphs are attracted to a warmed tube (Lees, 1948), and *Ixodes persulcatus* Schulze were found to migrate out of the leaf litter in response to a temperature gradient that was hotter at the soil surface (Alekseev and Dubinina, 2000). A positive thermotaxis is usually interpreted as behaviour that would increase the probability of attaching to a host animal.

A negative response to light has also been observed previously in mites, for example the Banks grass mite *O. pratensis* (Li and Margolies, 1991). In the present study the high degree of sensitivity to light shown by *Psoroptes* is particularly interesting since no organs capable of light detection have yet been identified. The tick, *Hyalomma truncatum* (Koch) shows movement towards dark stationary, two-dimensional objects with a greater response towards larger objects (Kopp and Gothe, 1995). The time taken for locomotion towards the object to begin is reduced in the presence of a carbon dioxide gradient. Although this response may be considered to be host-orientated behaviour, it was interpreted by these authors as a response to allow protection from harsh climatic conditions. This was because stationary objects were preferred to moving ones and the presence of a temperature gradient, mimicking the proximity of a host, did not enhance the response. This suggests that in addition to aiding host location, the various cues used by mites and ticks may also promote off-host survival. In the present study, since desiccation is an important mortality factor for *Psoroptes* mites off-host (Smith *et al.*, 1999), movement away

from light may also help mites move to locations with high relative humidity, thereby maximising off-host survival time. The results presented in this study show that although linear velocity of the mites was unaffected by the presence or absence of a uni-directional light gradient, angular velocity was greater when no uni-directional light gradient was present. Such an increase in turning may facilitate movement to areas of lower light intensity, where survival may be higher. Survival may also be increased at lower temperatures where the rates of energy use are reduced. In this study, mites were seen to move with decreased linear and angular velocity at lower temperatures, a behaviour that may act to retain energy reserves for reproduction and colonisation once a host has been located, where local temperatures will be greater.

For *Psoroptes,* the movement to areas of lower light intensity and higher temperature might be expected to help them to maintain their position on a host animal or help them locate the skin surface of a new host when displaced into the environment. On sheep, *Psoroptes* mites are found on the skin at the base of the fleece, an area where light intensity will be relatively low and temperature will generally be between 30 and 40°C (Wall *et al.*, 1992). Light cues may increase the rate and efficiency at which mites move to the base of the fleece thus allowing more rapid establishment and a greater success of transmission. In the present study, changes in activity with light or temperature gradients were continuous; at no point were step-changes in orientation or rate of movement observed. This suggests a graduated response to environmental cues is used to move *P. ovis* to areas of favourable conditions. A similar mechanism has been suggested by Li and Margolies (1991) in the location of the Banks grass mite on its host plant.

In many tick species, gravitational cues are used to ensure successful transmission to a new host and questing stages climb the vegetation to increase their

probability of contact with a potential host. Further to this negative geotaxis, it has been suggested that tick larvae may be able to detect their height above ground and form clumps at 50-190cm above ground level in the absence of external cues, even if the vegetation height is greater than this (McPherson *et al.*, 2000). The height at which the larvae aggregate corresponds to the torso heights of their hosts, therefore increasing the chance of transmission to the appropriate host. A similar response is also seen in phyophagous species of Acari. For example, the black-currant gall mite *Cecidophyopsis ribis* (Westwood) also shows a negative response to gravity (Herr, 1991). A climbing response was seen in the present study and such behaviour might be expected to bring off-host mites into positions where they would be more likely to make contact with a new host. However, the climbing response was largely abolished by the presence of strong illumination from above. The functional significance of this behaviour in *Psoroptes* is uncertain, but since these mites are highly susceptible to desiccation (Smith *et al.*, 1999), it may be that climbing is only attempted in darkness or conditions of low light intensity when humidity is likely to be relatively high.

The data presented here support the suggestion that there may be a hierarchy among stimuli used by these parasites to find a new host (MacInnis, 1976). The light cues modified the temperature-gradient information and both effectively abolished the climbing response. MacInnis also suggested that there may be synergistic effects among stimuli and this was also observed here; when exposed to gradients of both temperature and light, mites moved greater distances than when either cue was present alone.

CHAPTER 5

OFF HOST SURVIVAL OF *PSOROPTES* MITES

5.1 Introduction

The off-host survival time of *Psoroptes* mites has been examined in several studies previously. Estimates range from several days (O'Nuallain, 1966) to several years (Salmon & Stiles, 1903; Dill, 1920). The primary importance of such work is to determine the time for which enclosures that have previously housed infected individuals must be left vacant, to prevent the subsequent infection of naïve or uninfested individuals. However, off-host survival times have also been examined in the laboratory as part of investigations into the feasibility of rearing of mites *in vitro*. *Psoroptes* mites currently can only be reared on a live vertebrate host. An *in vitro* colony would provide a valuable source of mites for experiments into the control of the disease. This would replace the need to maintain infested living hosts and would therefore be of animal welfare value and would also be more cost-effective, since artificial colonies would be less expensive and labour intensive to maintain, especially given the large numbers of mites that may be necessary for control trials.

To rear *Psoroptes* mites *in vitro*, it is of great importance that the optimum conditions required by the mite are determined. One way of identifying such conditions in the first instance is by maximising off-host survival.

Several studies have examined the effect of temperature on the off-host survival time of the mites (Arlian *et al.*, 1981; Liebisch *et al.*, 1985; Smith *et al.*, 1999, Meintjies *et al.*, 2002c). All of these studies conclude that higher temperatures reduce mite longevity, with maximum survival recorded ranging from 15 days when maintained at 9°C (Smith *et al.*, 1999) to 48 days when kept at 5 or 10°C (Liebisch *et*

al., 1985). However, as suggested by Hayes and Wall (1999), it may be more appropriate to consider physiological longevity as opposed to chronological longevity as this would allow more accurate comparison of mite longevity at different temperatures. Mites living for the same physiological time would be expected to survive for longer at lower temperatures when measured chronologically as a simple physiological response to lower temperatures causing reduced rates of metabolism. Furthermore, maintenance of mites at low temperatures would be expected to adversely affect egg production (Mathieson, 1995).

None of the previous studies of survival have looked at the effect of temperatures above 30°C, despite the fact that the temperature of a sheep at the base of the fleece may be between 30 and 40°C (Wall *et al.*, 1992; Mathieson, 1995). Wilson *et al.* (1977) concluded that high temperatures are detrimental to mite survival as no mites were able to survive for longer than 9 days when kept at 37°C and Arlian *et al.* (1981) observed 100% mortality in mites from rabbits that were maintained at 40°C and 20% r.h. for 24 hours. Although again, the note about the importance of physiological age also applies here.

Humidity has also been shown to have an effect on mite longevity. Smith *et al.* (1999) showed that below a humidity threshold of 65-75% r.h., when temperature is maintained at 30°C, the LT_{50} survival times of mites were significantly lower. Mathieson (1995) also found that low humidity was detrimental to mite survival. It has also been suggested by Babcock and Black (1933) that increased humidity increases the rate of egg deposition and that rainfall and high ambient humidity are amongst the main factors responsible for changes in the incidence of sheep scab (Shilston, 1915).

Most laboratory studies have examined the survival un-fed of *P. ovis*, as off-host feeding has proven difficult, and it has not yet been possible to create a successful sustainable *in vitro* feeding system. DeLoach (1984) fed mites using a

glass chamber and mesh nylon netting through which the mites could feed. He determined whether the mites had fed by observing colour change from white to red when blood cells or red dye placed in the food were ingested. Although approximately 85% of female mites that had been kept off the host for one day fed, DeLoach was unable to keep the mites alive for any longer than 7 days. He concluded that feeding did not enhance off-host survival. Mathieson (1995) looked at four different devices for *in vitro* feeding: a sheep blood agar plate; a petri-dish gauze feeder, comprising of a piece of filter paper soaked in feeding solution covered with nylon gauze and enclosed within a petri-dish; a Perspex feeding cylinder, where mites feed through nylon gauze placed on a rubber gasket over a reservoir filled with feeding solution; and a plexi-glass feeding device, where a feeding solution soaked piece of filter paper was sandwiched between two pieces of plexi-glass with holes drilled in to create a chamber and covered with a glass coverslip. Some success was achieved with the latter two devices, with mites surviving for longer than unfed mites. However, mites still failed to survive as long as those on-host.

Many practical problems have been encountered in the development of feeding systems. These have included the fact that high humidity inside the feeding chamber may congeal the feeding medium. Mathieson noted that egg production was much reduced compared to what would be expected on host, and that larvae died quickly after hatching. From this he concluded that the diet provided (sheep blood agar, whole defibrinated sheep blood and sheep plasma) may not have contained enough nutrition or the mites may not have been feeding to repletion.

As well as maximising mite off host survival, some authors have considered it crucial to determine the conditions which enhance oviposition and the development of eggs, larvae and nymphal stages. Although ovigerous female mites are thought to lay 2.9 eggs per day (Downing, 1936b; Wall *et al.*, 1999), oviposition rates have been observed to be much lower *in vitro* and in some studies cease all together

following removal from the host (Stockman and Berry, 1913), with no females seen to lay eggs when they have been removed from the host for more than 2 days (Shilston, 1915). When incubation is continued after eggs have been laid, Stockman and Berry (1913) found that *in vitro* hatching took 100 h, although the temperature at which the eggs were incubated was not noted in that study. Shilston (1915) found that on the host or when incubated at 37°C, eggs will hatch within 2-3 days and Downing (1936b) found that when on the host, eggs hatch in an average of 2.7 days (Wall *et al.*, 1999). However, Meintjes *et al.* (2002c) collected eggs from the host and unusually found mean hatch times of up to 22.14 days, with hatch times, as expected, being generally longer at lower temperatures. When maintained at 90% r.h., the mean time required for eggs to hatch was 17.5 days at 10°C and 7.43 days at 25°C (Meintjes *et al.*, 2002c). The mean maximum survival time of larvae resulting from these eggs was found to be 9.25 days, with a general trend of longevity decreasing with increasing temperatures. Again, when maintained at 90% r.h. larvae survived for 9.25 days at 10°C and 5.17 days at 25°C. On-host, it is thought that the duration of the larval stage is approximately 2-3 days (Downing, 1936b; Wall *et al.*, 1999).

The aim of the work described in this chapter was to extend the previous studies of the optimum temperature and diet for *Psoroptes* mites, to not only maximise off-host survival but also optimise oviposition and the rates of development of egg, larval and nymphal stages.

5.2 General materials and methods

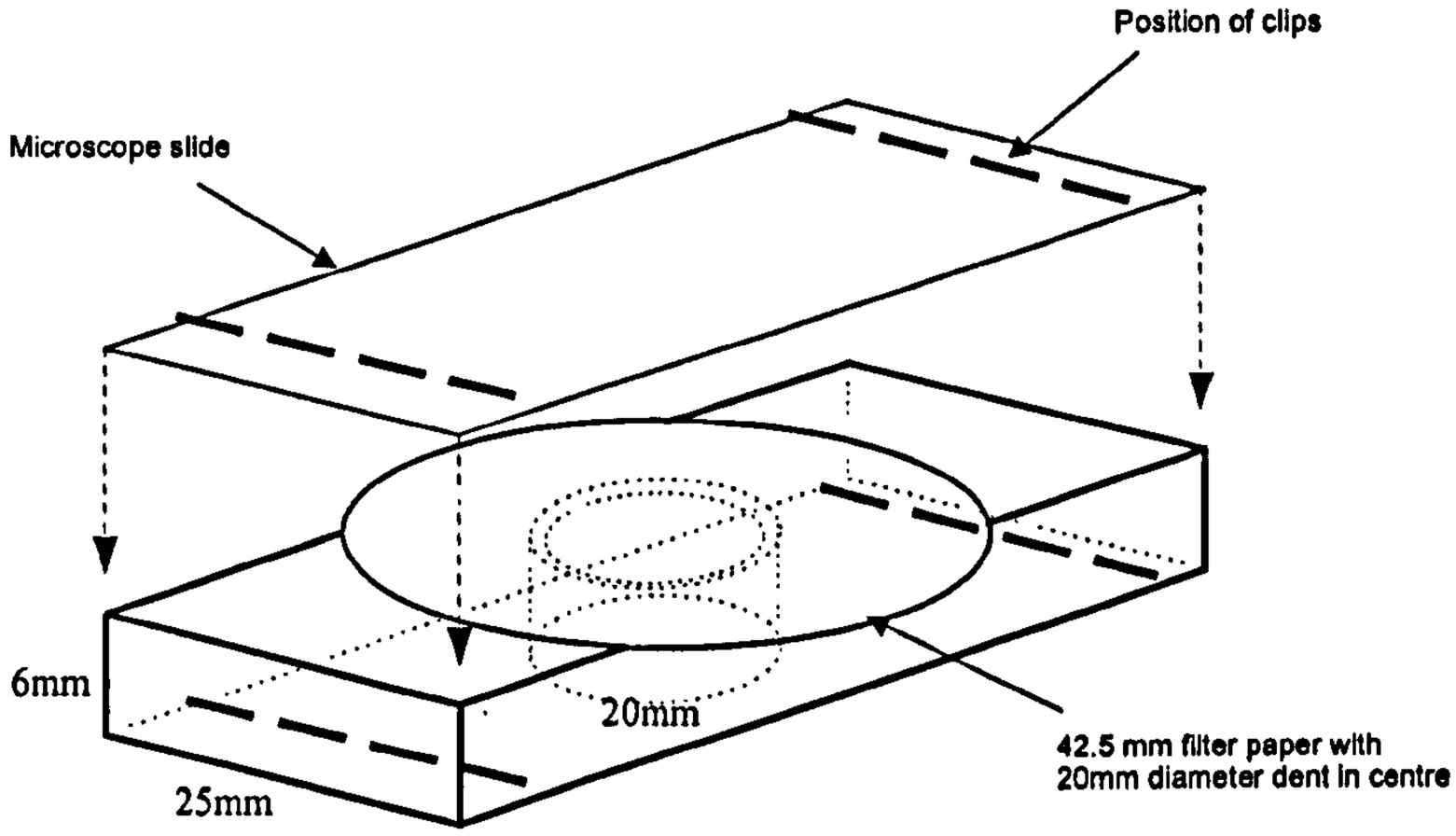
Apparatus and experimental design

To monitor the off-host survival and development of *Psoroptes* mites, a standardised bioassay was developed. Several approaches were investigated; the

design finally selected for use was adapted from that described by Smith *et al.* (1999). A chamber was constructed from a 25 x 75 x 6 mm glass block in which a central 20 mm diameter hole had been drilled (Fig. 5.1). An indented circle of filter paper (Whatman International Ltd, Maidstone, UK) covered this hole and the various *Psoroptes* life cycle stages could be placed onto the filter paper. The filter paper was then covered with a microscope slide to enclose the mites and the block and slide were held together by two fold back clips at either end.

The work presented in this chapter describes the examination of adult females, larvae and eggs. Adult males or nymphs were not considered here. Chambers containing either five adult females or a single egg or larva were maintained in an incubator (Sanyo Gallenkamp Plc, Loughborough, UK. Model No. MLR-350H) in which the temperature could be varied and where humidity was maintained at above 90% r.h. Adult female mites had been off host for no longer than 1 h when placed into a chamber. Liquid sources of nutrition were added in 200µl quantities daily via the base of the chamber and allowed to soak into the filter paper disc. Any solid food sources were added directly onto the filter paper inside the chamber.

Figure 5.1 Bioassay chamber for mite survival analysis. Adapted from Smith *et al.* (1999).



During trials the chambers were examined twice daily and the number of live mites was counted. Death of a mite was identified by the absence of movement following mechanical stimulation with a fine paint brush. Any dead adult females were removed from the chamber and all live mites were transferred into a clean chamber daily. Any eggs were counted and placed individually into a separate chamber where they were incubated under the same conditions as the adult female that had laid them. Observations of adult female mites continued until all the mites in the chamber were dead. Chambers containing eggs were inspected twice daily so that approximate time of hatching could be determined. For larvae, observations continued for at least two days after death because death was more difficult to determine.

Analysis

For adult female mites, LT_{50}, maximum survival time, oviposition rate and maximum period of oviposition were calculated for each chamber. The LT_{50} value was defined as the time taken to reach 50% mortality and calculation of this value assumed that a linear mortality rate occurred between inspections. Maximum survival time was measured as the maximum longevity within a chamber. Oviposition rate was calculated as the mean number of eggs laid per adult female mite in the first 24 hours off-host. Maximum period of oviposition was estimated as the maximum time that any mite in a single chamber was able to continue to lay eggs following removal from the host. For eggs, the time take to hatch was recorded and for larvae, survival time was noted and used for further analysis.

For all statistical analyses, the normality of the data was confirmed using a 1-sample K-S test and Levene's test was used to check for homogeneity of variances.

5.3 Effect of temperature

5.3.1 Materials and methods

To examine the effect of temperature on the survival and rate of development of the various life-cycle stages, all stages were incubated at either 25, 27.5, 30, 32.5, 35, or 37.5°C. For each of the temperatures, mites were either maintained on rabbit serum (Sigma-Aldrich, Poole, UK), or one of two control treatments, of distilled water (control 1) or no addition to the chamber (control 2).

For the adult female mites fully factorial ANOVA were carried out with LT_{50}, maximum survival, oviposition rate and maximum period of oviposition as the dependent variable and with temperature and diet treatment as fixed factors. The percentage of eggs that hatched successfully, the time required for eggs to hatch and the survival time of larvae were also put into a factorial ANOVA as dependent variables also with temperature and diet treatment as fixed factors. Tukey post-hoc tests were used to explore variation in the data between treatment groups. Larval survival time was regressed against temperature.

5.3.2 Results

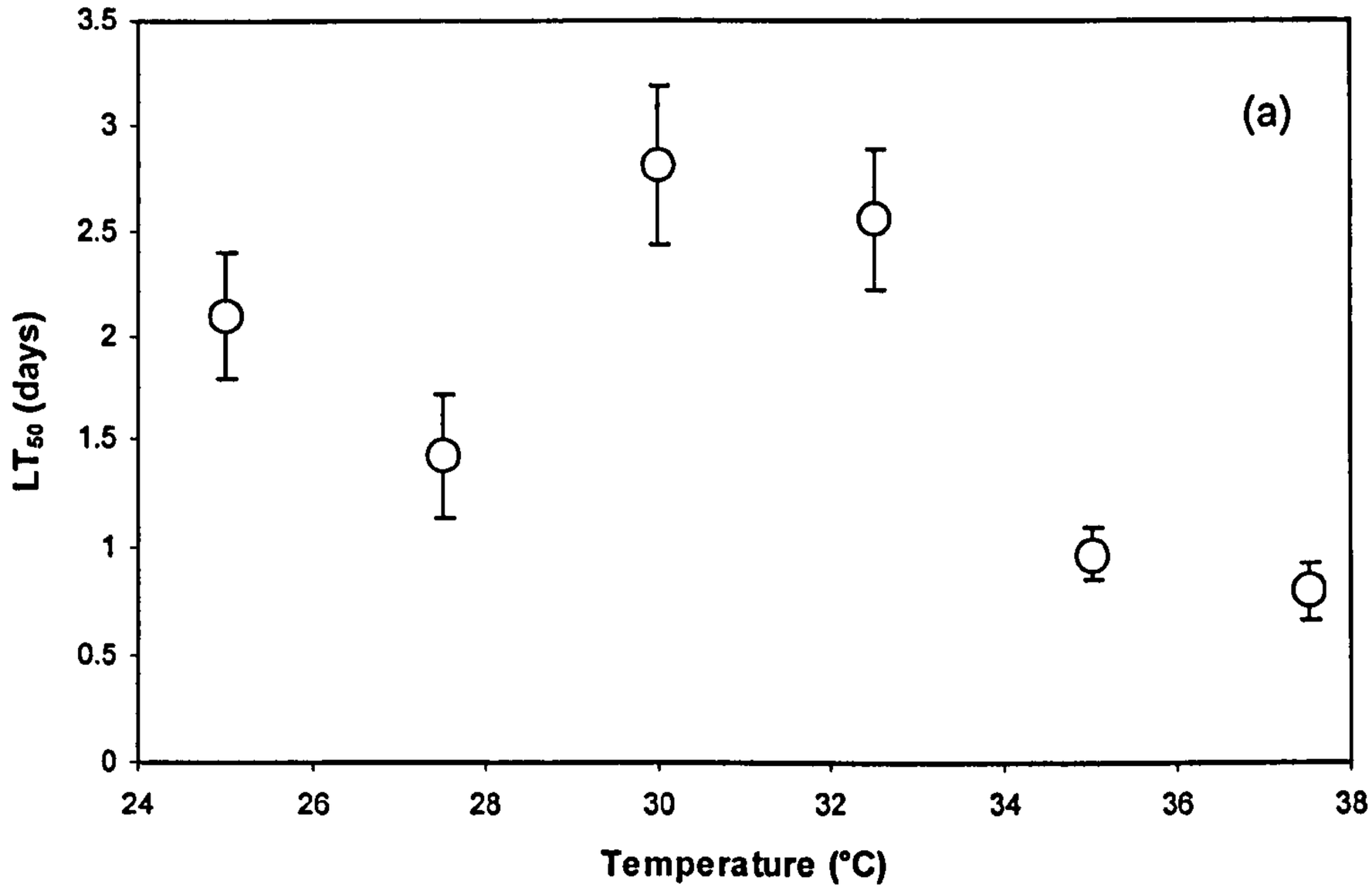
Both temperature and diet had a significant effect on the LT_{50} of adult female mites ($F_{5,147}$=11.82, P<0.001 and $F_{2,147}$=11.06, P<0.001 respectively; Fig. 5.2). LT_{50} was greatest at 30°C with a reduction in LT_{50} at temperatures higher and lower than 30°C. Tukey post-hoc tests showed that LT_{50} is significantly greater in mites maintained at 30°C than those maintained at 27.5, 35 or 37.5°C but there are no significant differences between mites maintained at 25, 30 or 32.5°C. The LT_{50} values of mites maintained on rabbit serum were significantly greater than either of the control treatments. There was a significant interaction between temperature and diet ($F_{9,147}$=4.71, P<0.001). The LT_{50} of starved mites (control 2) occurred at a

lower temperature to mites under the other two treatments. Maximum LT_{50} occurred at 30°C for starved mites and 32.5°C for mites given rabbit serum or distilled water (Figure 5.3).

A similar pattern was seen in the maximum survival of adult female mites. There was a significant effect on maximum survival of the mites by temperature ($F_{5,147}$=12.18, P<0.001; Fig. 5.4a) and diet ($F_{2,147}$=13.10, P<0.001; Fig. 5.4b). Mites maintained at 30°C had significantly longer maximum survival than those maintained at 27.5, 35 and 37.5°C, but maximum survival was not significantly different between mites maintained at 25, 30 or 32.5°C. Tukey post-hoc tests showed that the maximum survival of mites given rabbit serum was significantly greater than mites provided with either of the control treatments. There was a significant interaction between the two factors ($F_{9,147}$=3.51, P=0.001). Maximum survival reached its peak at 30°C for starved mites (control 2) and at 32.5°C for mites given rabbit serum or distilled water (control 1) (Fig. 5.5).

Oviposition rate was significantly affected by both temperature of incubation ($F_{5,147}$=12.92, P<0.001; Fig. 5.6a) and the diet with which mites were provided ($F_{2,147}$=31.87, P<0.001; Fig. 5.6b). Maximum oviposition rate occurred when mites were incubated at 30°C and oviposition rate at this temperature was significantly greater than that of mites incubated at 25, 27.5, and 35°C. There was no significant difference in oviposition rate between mites maintained at 30, 32.5 or 37.5°C. There was a significant difference between the number of eggs laid by females given different diets, with mites maintained on rabbit serum laying more eggs than mites maintained on either of the control diets. There was a significant interaction between temperature and diet ($F_{9,147}$=3.88, P<0.001). Mites given rabbit serum showed maximum rate of oviposition from 30-32.5°C, at a larger range of temperatures than either of the control treatments. Starved mites (control 2) had maximum oviposition rate at 30°C and mites given distilled water (control 1) at 32.5°C. (Fig. 5.7).

Figure 5.2 Mean LT_{50} (±s.e.) for adult female *Psoroptes ovis* mites (a) when incubated at a range of temperatures and (b) when maintained on rabbit serum or distilled water or starved.



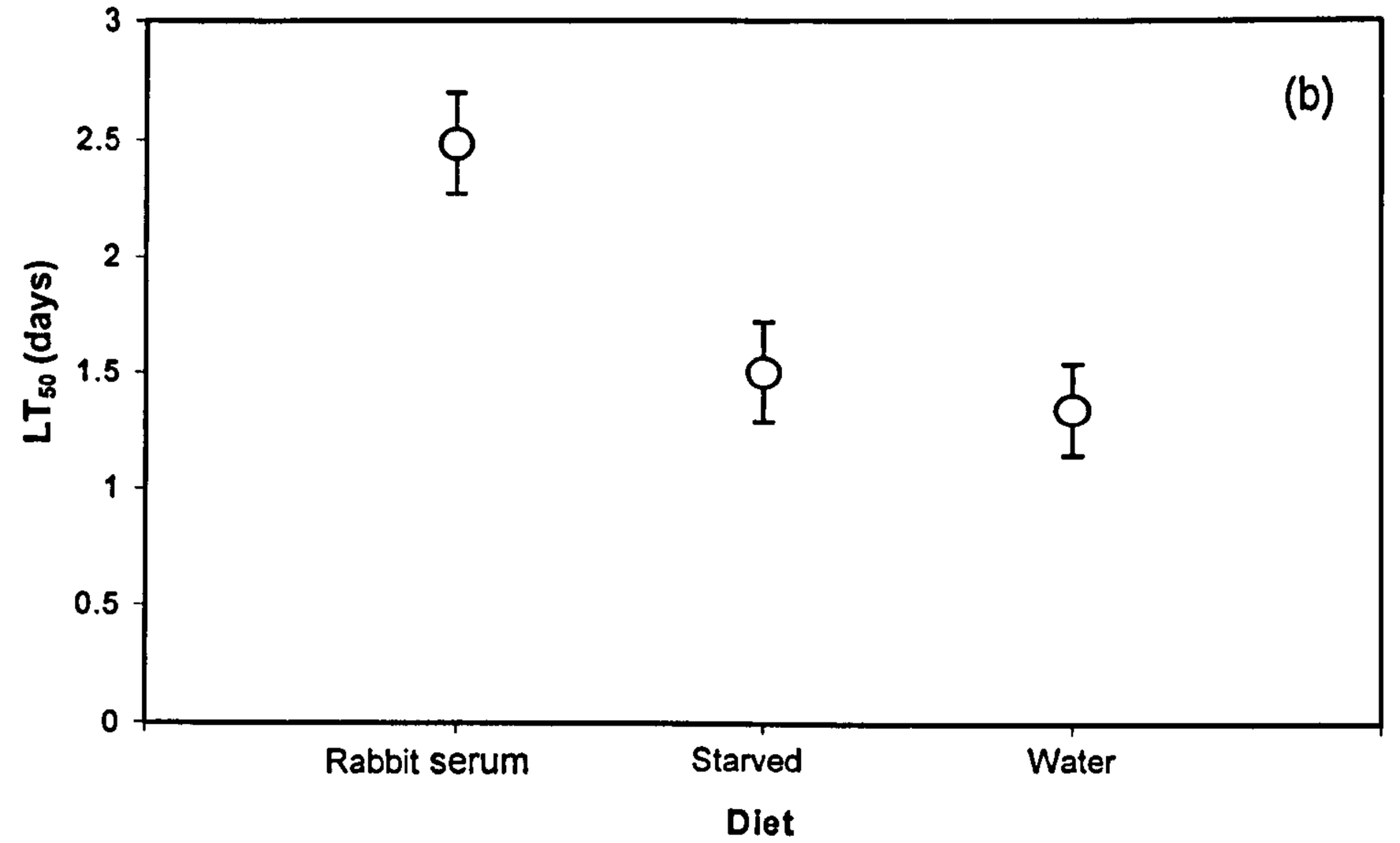


Figure 5.3 Mean LT_{50} (±s.e.) for adult female *Psoroptes ovis* mites maintained on rabbit serum or distilled water or starved and incubated at a range of temperatures. Points joined for clarity.

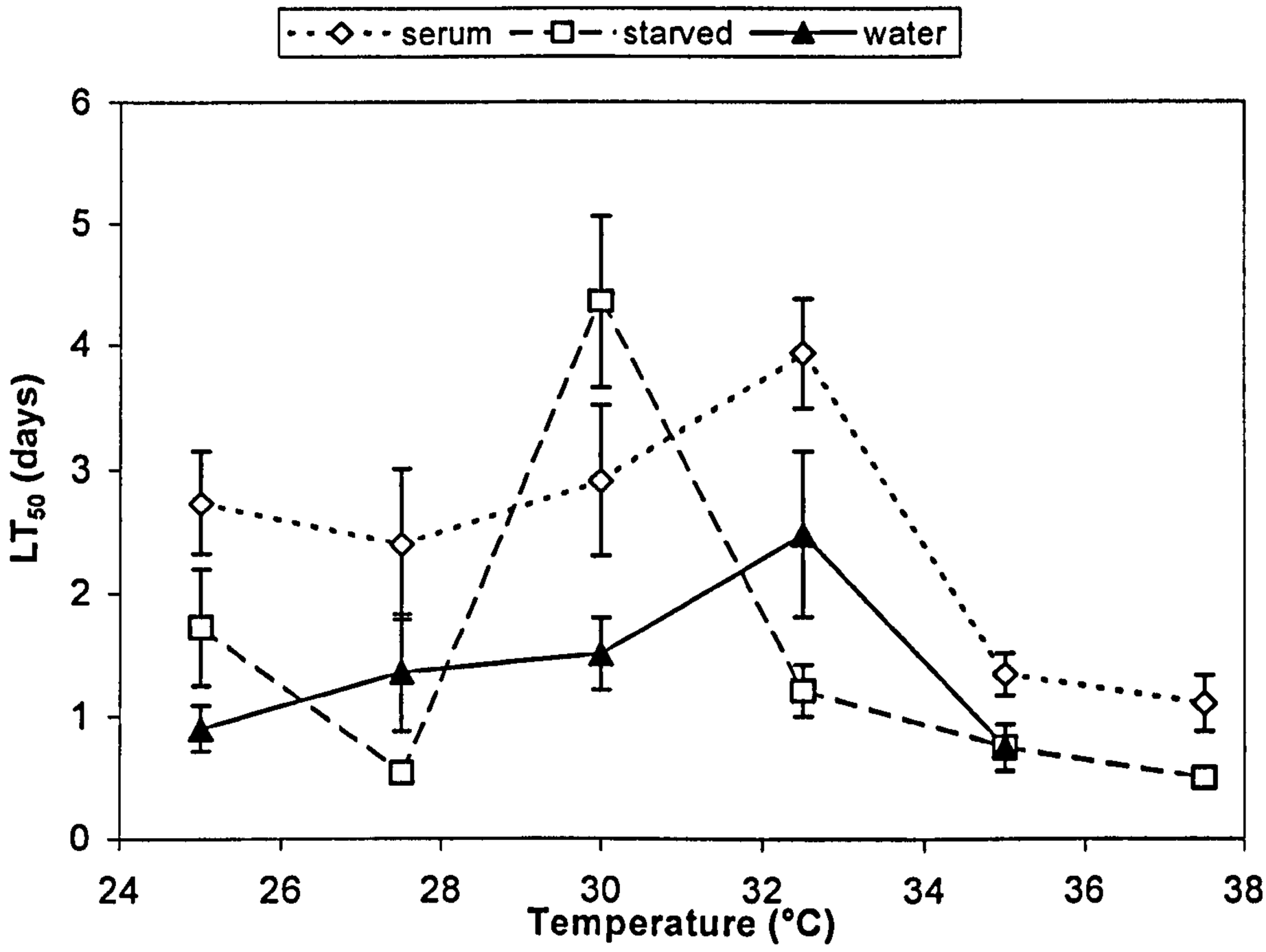
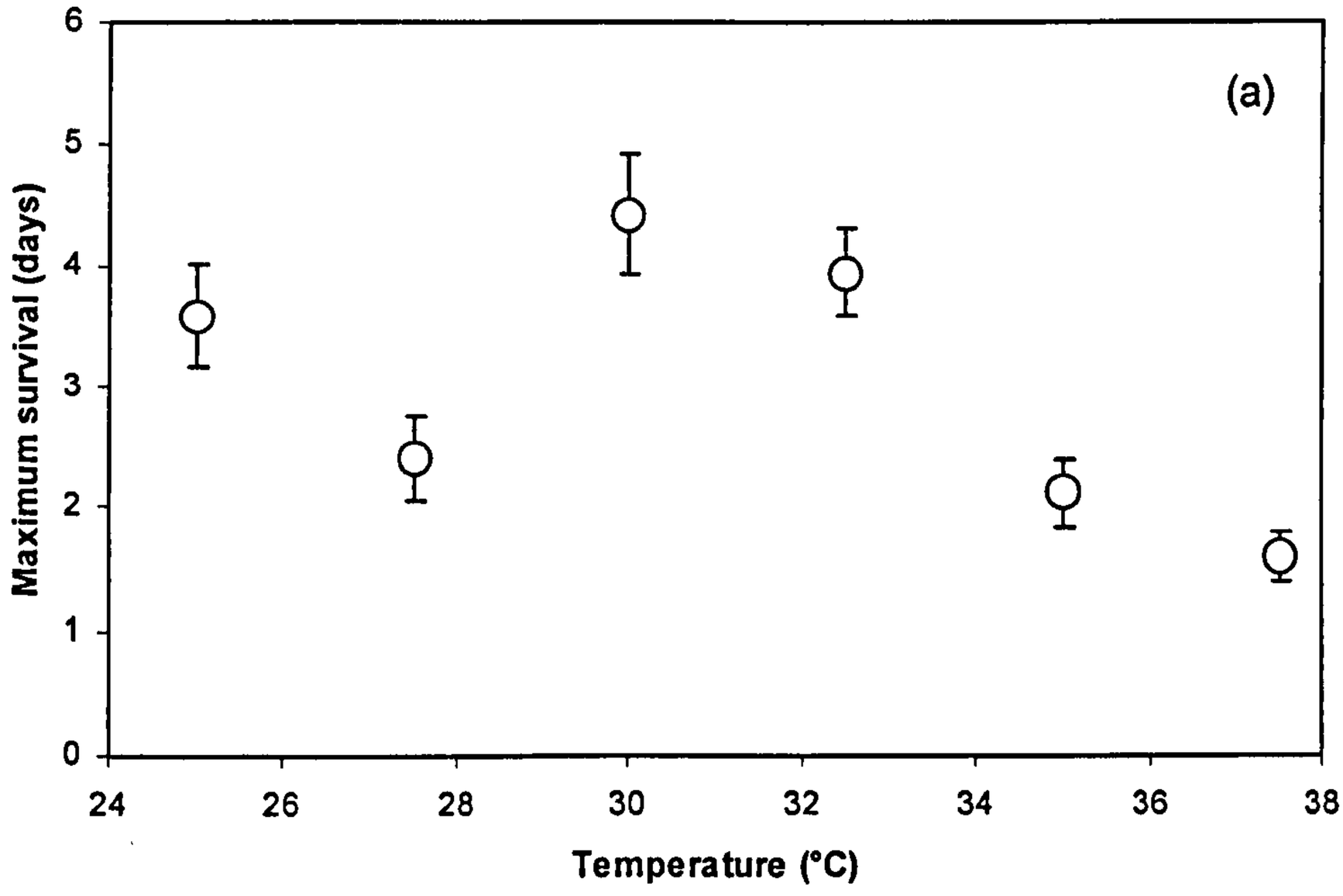


Figure 5.4 Mean maximum survival (±s.e.) for adult female *Psoroptes ovis* mites (a) when incubated at a range of temperatures and (b) when maintained on rabbit serum or distilled water or starved.



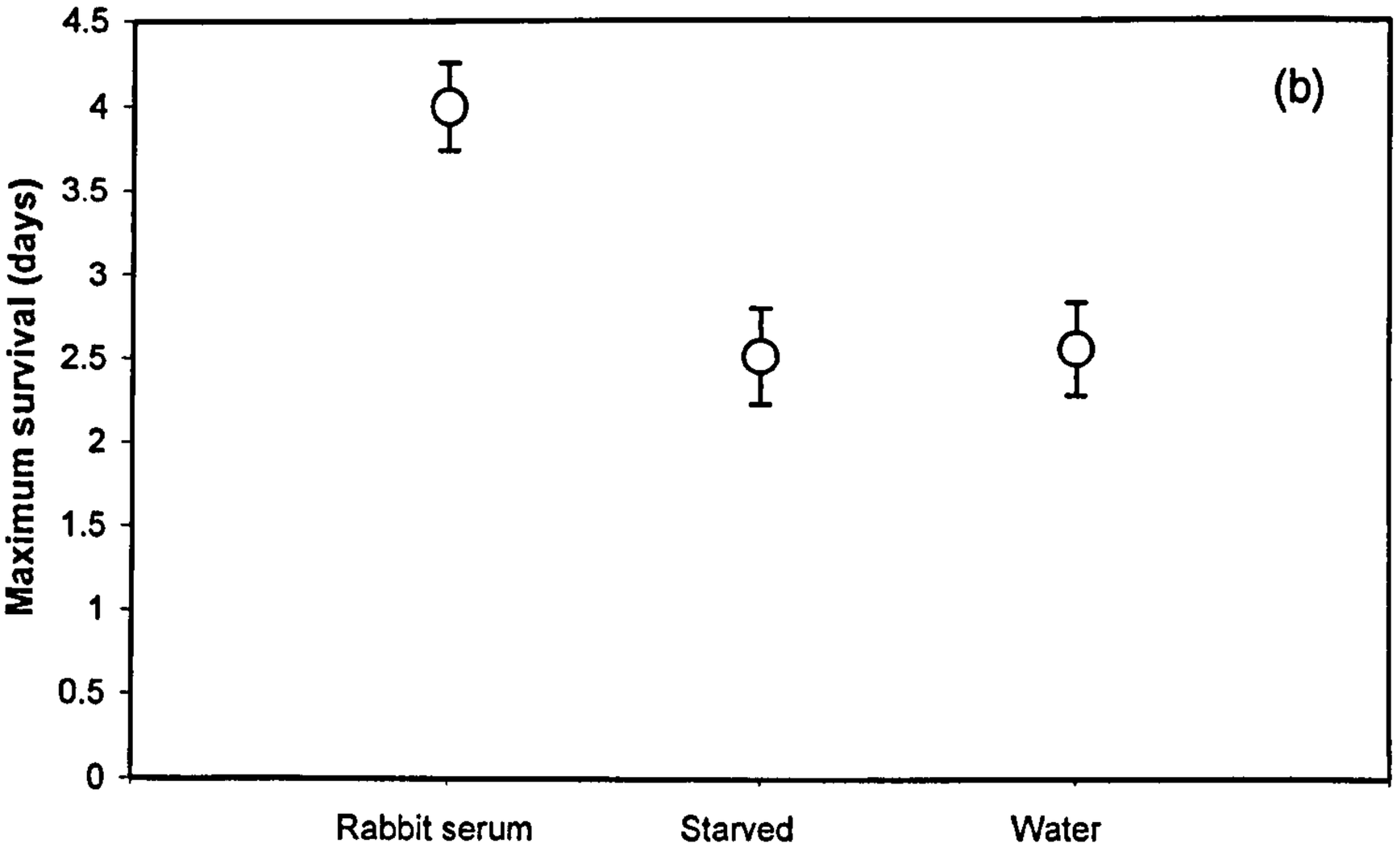


Figure 5.5 Mean maximum survival (±s.e.) of adult female *Psoroptes ovis* mites maintained on rabbit serum or distilled water or starved and incubated at a range of temperatures. Points joined for clarity.

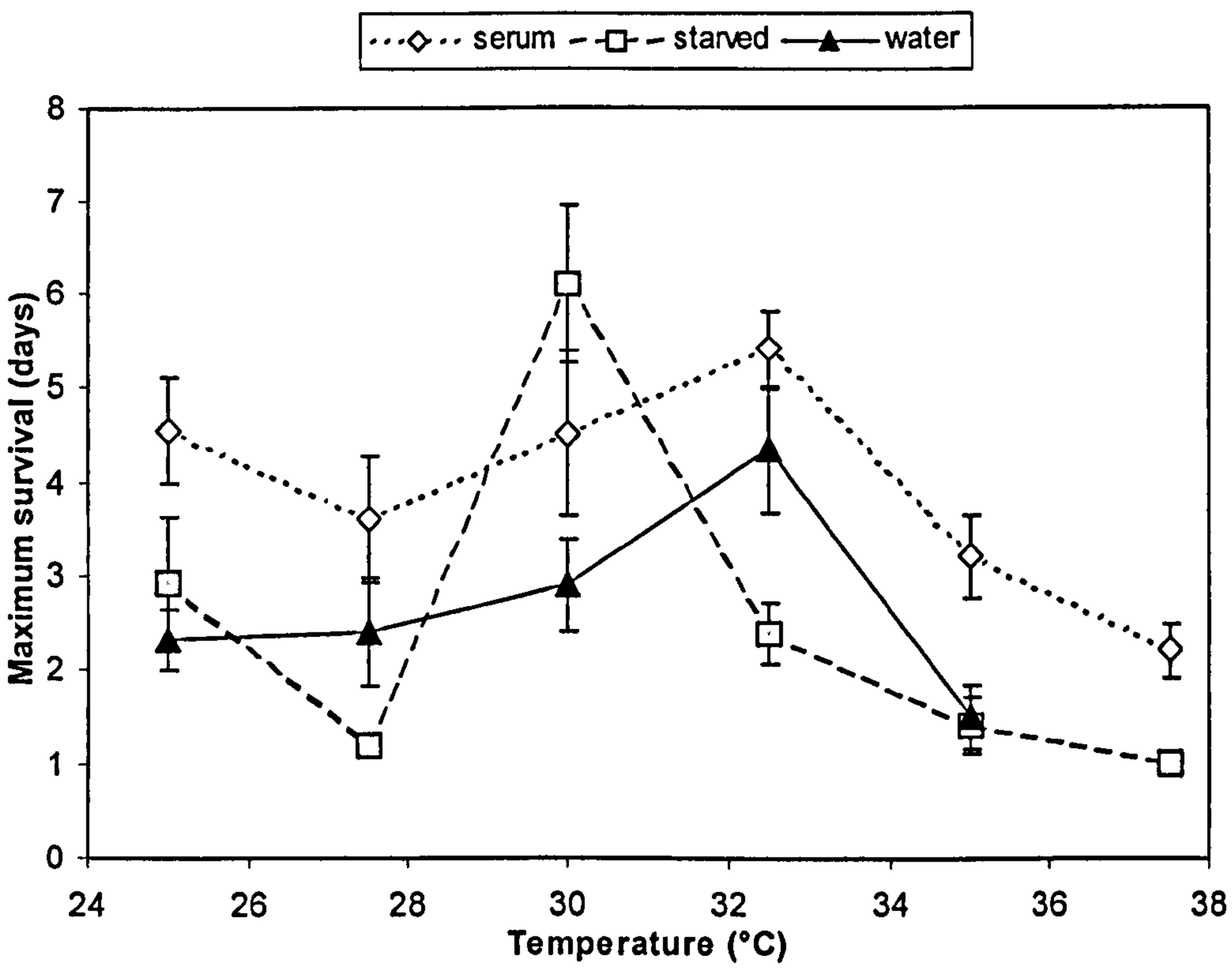
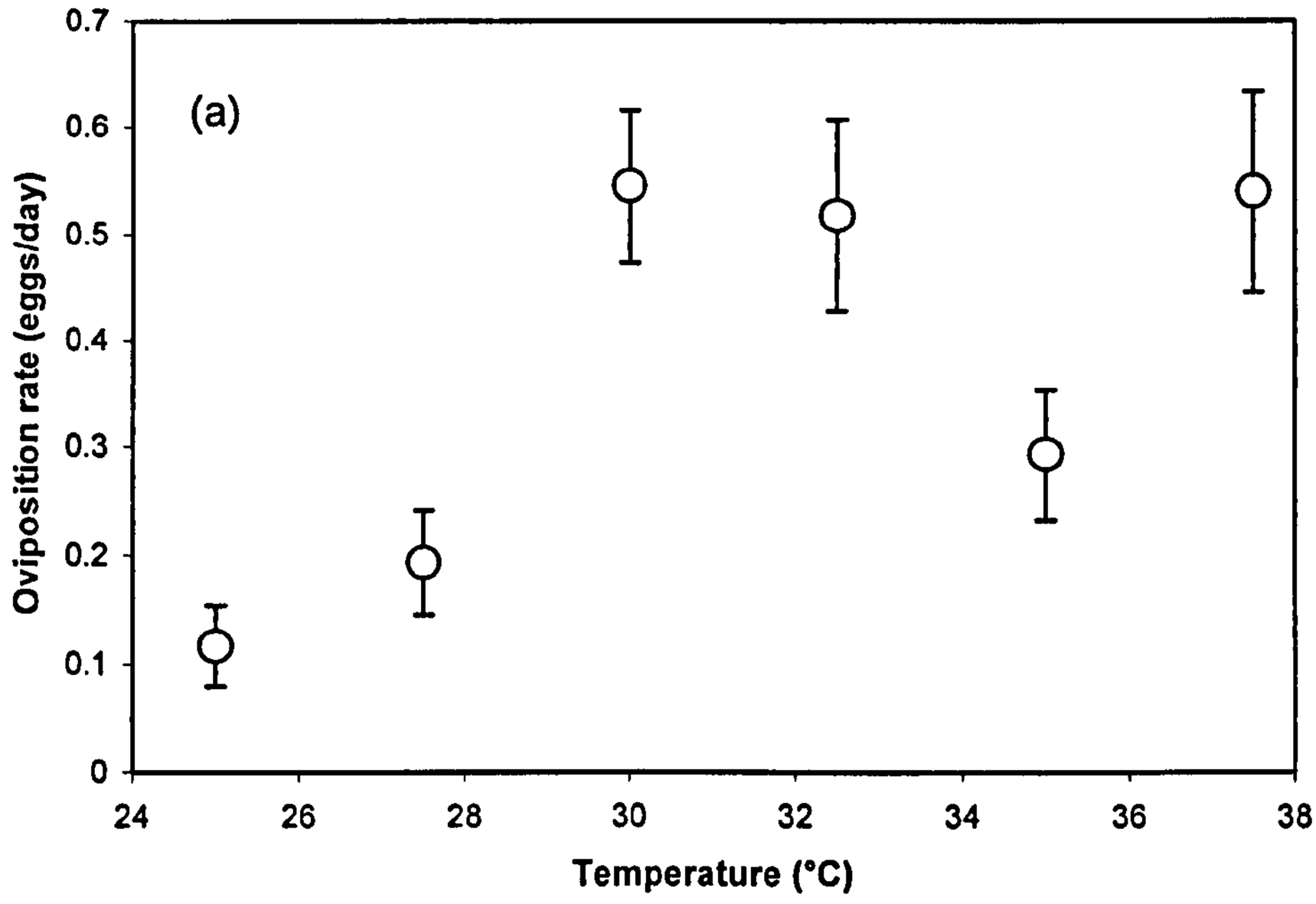


Figure 5.6 Oviposition rate (±s.e.) for adult female *Psoroptes ovis* mites (a) when incubated at a range of temperatures and (b) when maintained on rabbit serum or distilled water or starved.



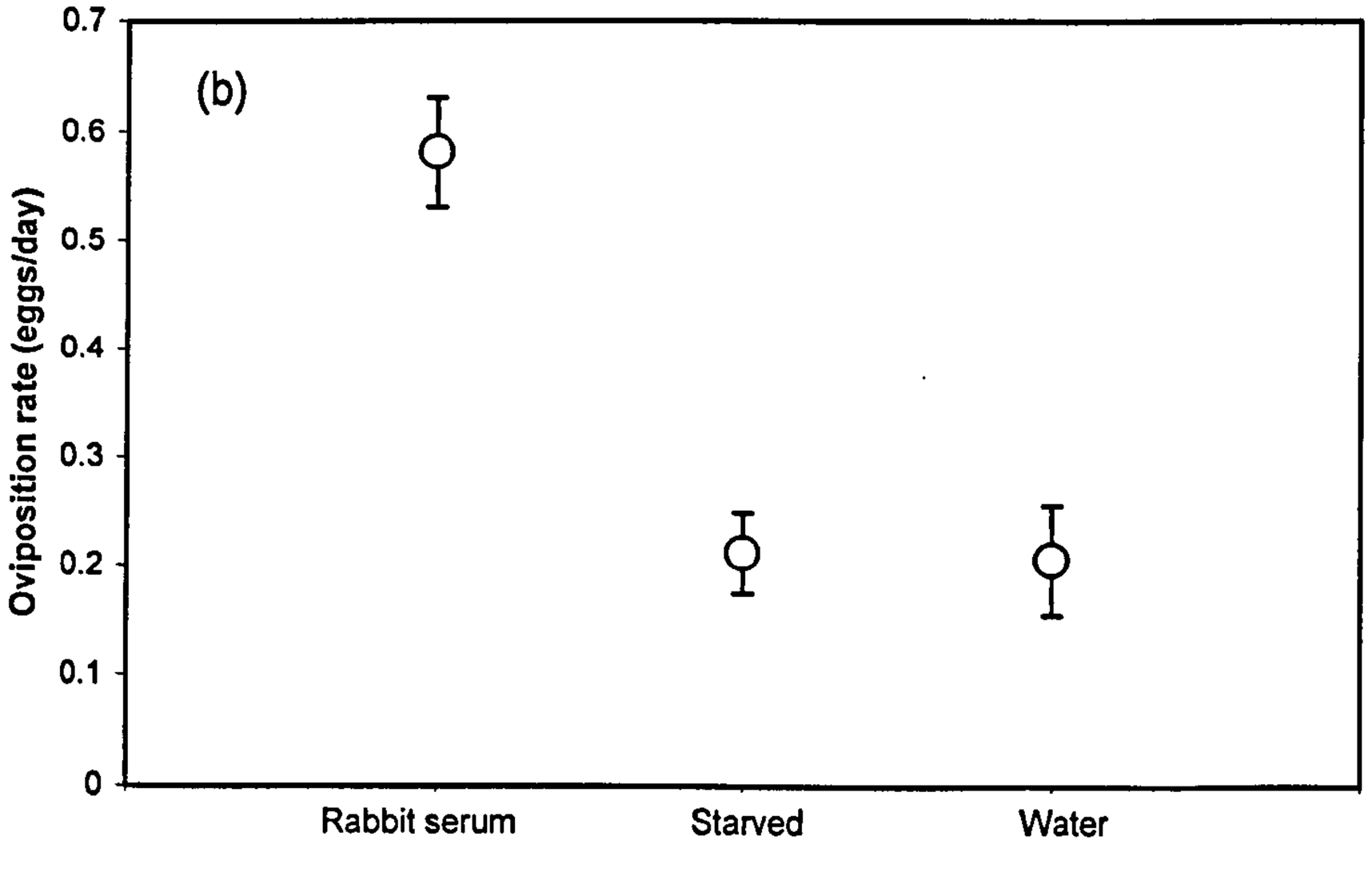
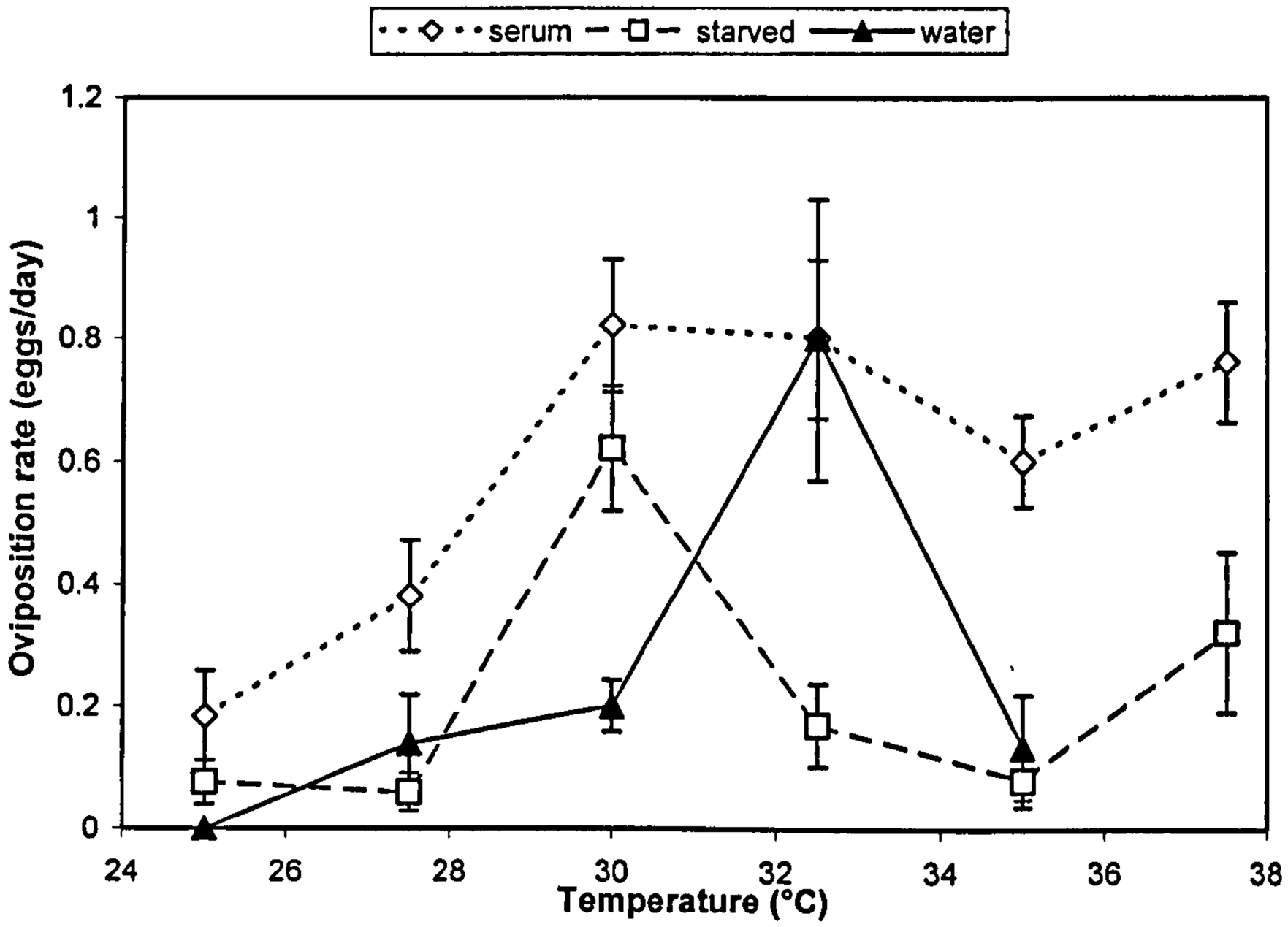


Figure 5.7 Oviposition rate(±s.e.) of adult female *Psoroptes ovis* mites maintained on rabbit serum or distilled water or starved and incubated at a range of temperatures. Points joined for clarity.



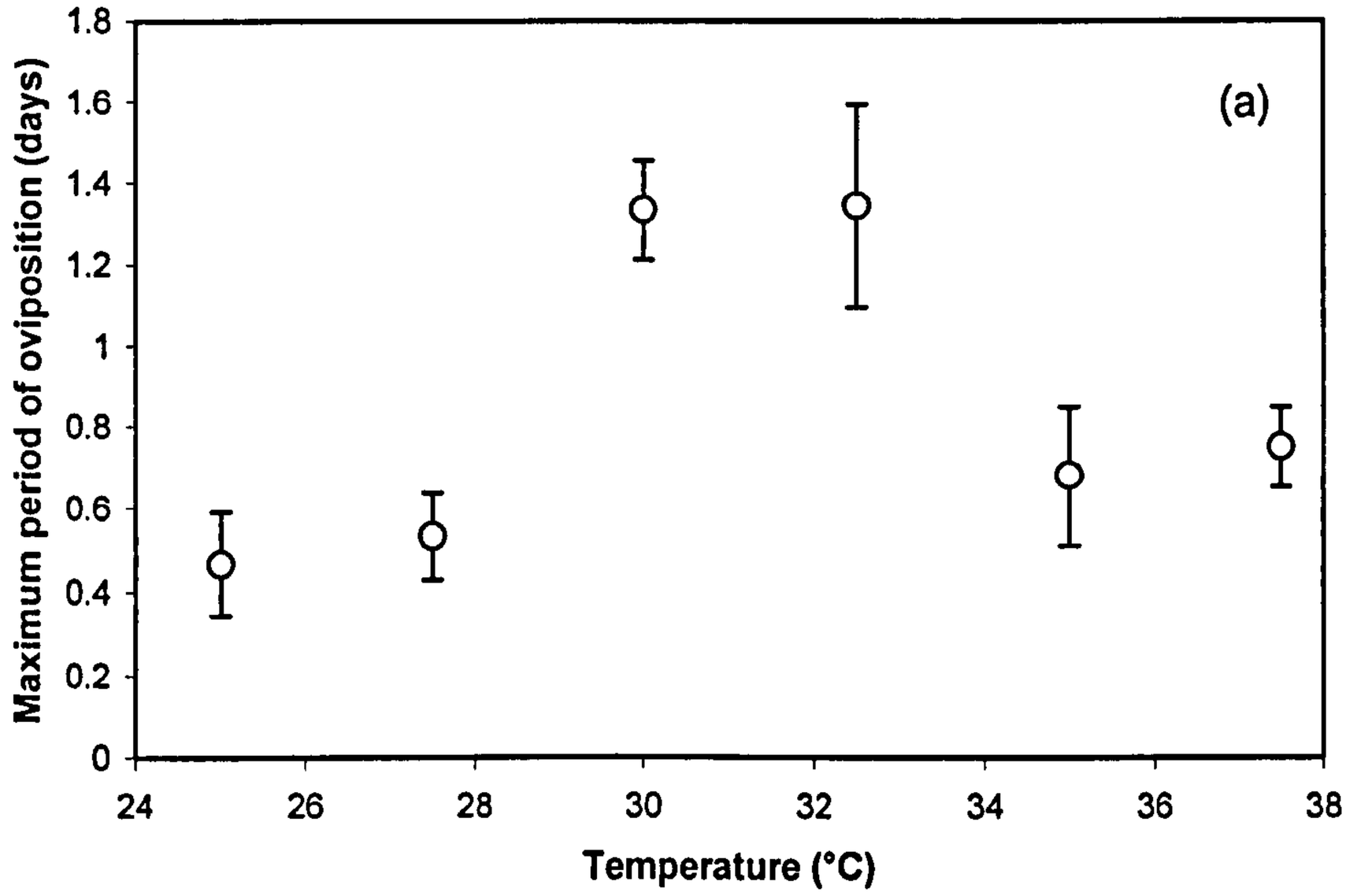
The maximum period of oviposition was significantly affected by temperature and diet ($F_{5,147}$=8.07, P<0.001 and $F_{2,147}$=13.29, P<0.001, respectively; Fig. 5.8a,b). The maximum period of oviposition occurred when mites were maintained at 32.5°C and period of oviposition at this temperature was significantly longer than in mites maintained at 25, 27.5 and 35°C. Period of oviposition did not differ significantly between mites maintained at 30, 32.5 or 37.5°C. Tukey post-hoc tests show that mites maintained on rabbit serum had a significantly greater maximum period of oviposition than those maintained under either of the control treatments. There was a significant interaction between temperature and diet ($F_{9,147}$=3.54, P=0.001). Mites maintained on rabbit serum were able to continue to lay eggs for the maximum amount of time when incubated at 32.5°C where maximum lay time of mites maintained on distilled water or starved occurred at 30°C (Fig. 5.9).

The percentage of eggs that hatched successfully ranged from 32%, when adult female mites and eggs were maintained at 25°C on rabbit serum, to 100% when adult females and eggs were maintained at 30°C on rabbit serum. Both of the control treatments also gave 100% hatch success when incubated at 30°C (control 1; Fig. 5.10) or at 32.5°C (control 2; Fig. 5.10). However, the differences observed in hatch success rate under different temperature and diet treatments were not statistically significant.

The time taken for eggs to hatch varied significantly with both temperature ($F_{5,198}$=322.80, P<0.001)(Fig. 5.11a) and diet ($F_{2,198}$=17.84, P<0.001)(Fig. 5.11b) with the time required when adult females and eggs were starved (control 2) being significantly greater than for either of the other diets. There was a significant interaction between temperature and diet ($F_{7,198}$=3.18, P=0.003) with diet having a greater effect on time to hatch at lower temperatures (Fig. 5.12).

The survival time of larvae varied significantly with temperature ($F_{5,198}$=9.14, P<0.001) but the diet with which the larvae were provided did not have a significant effect on survival. No larvae were observed to complete their developmental stage and moult into protonymphs. When data for all three diets were pooled, larval survival had a weak but significant linear relationship with temperature ($F_{1,211}$=32.40, P<0.001, r^2=13.3%; Fig. 5.13). The time for which larvae were able to survive was lower at higher incubation temperatures.

Figure 5.8 Mean maximum period of oviposition (±s.e.) of adult female *Psoroptes ovis* mites (a) when incubated at a range of temperatures and (b) when maintained on rabbit serum or distilled water or starved.



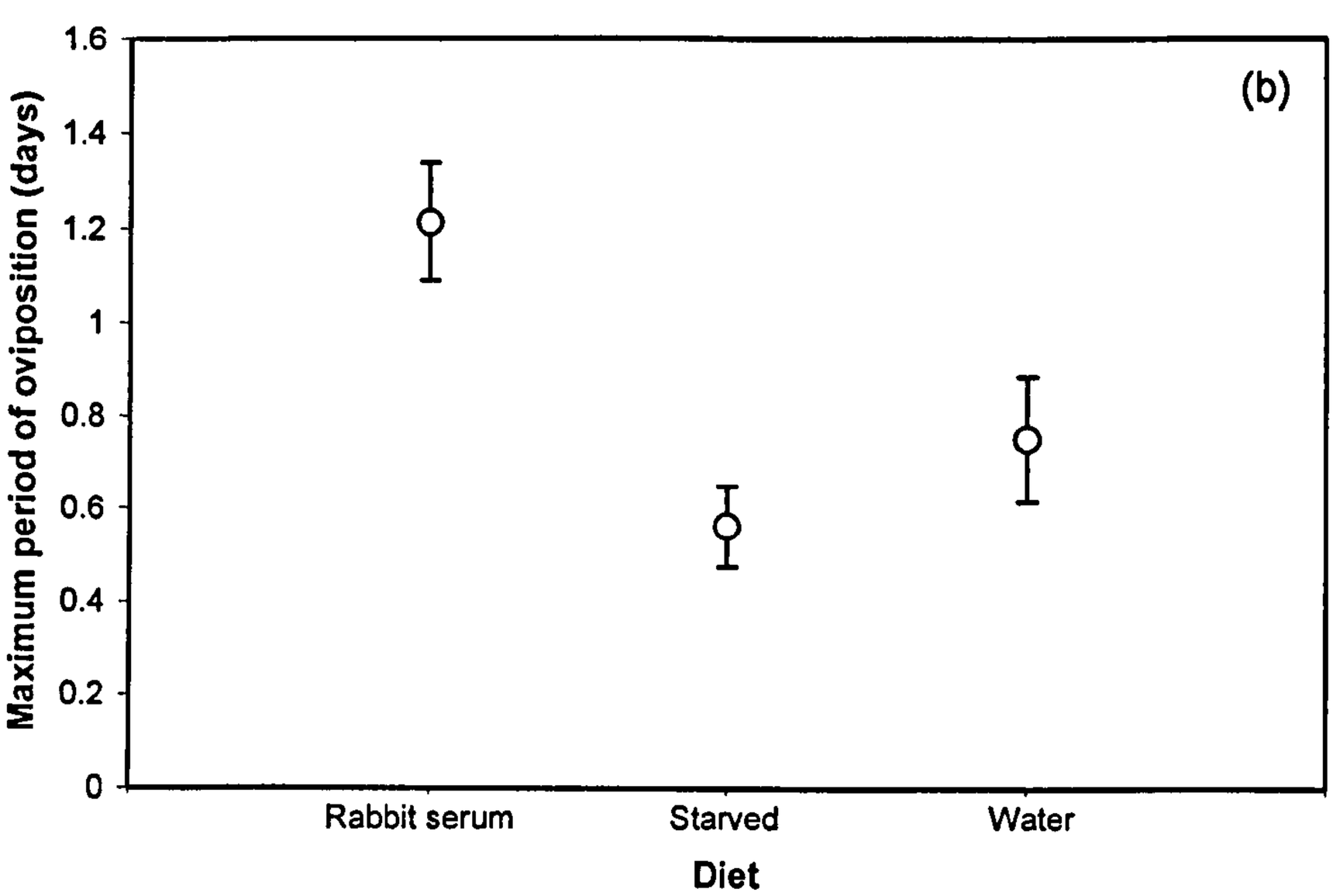


Figure 5.9 Mean maximum period of oviposition (±s.e.) of adult female *Psoroptes ovis* mites maintained on rabbit serum or distilled water or starved and incubated at a range of temperatures. Points joined for clarity.

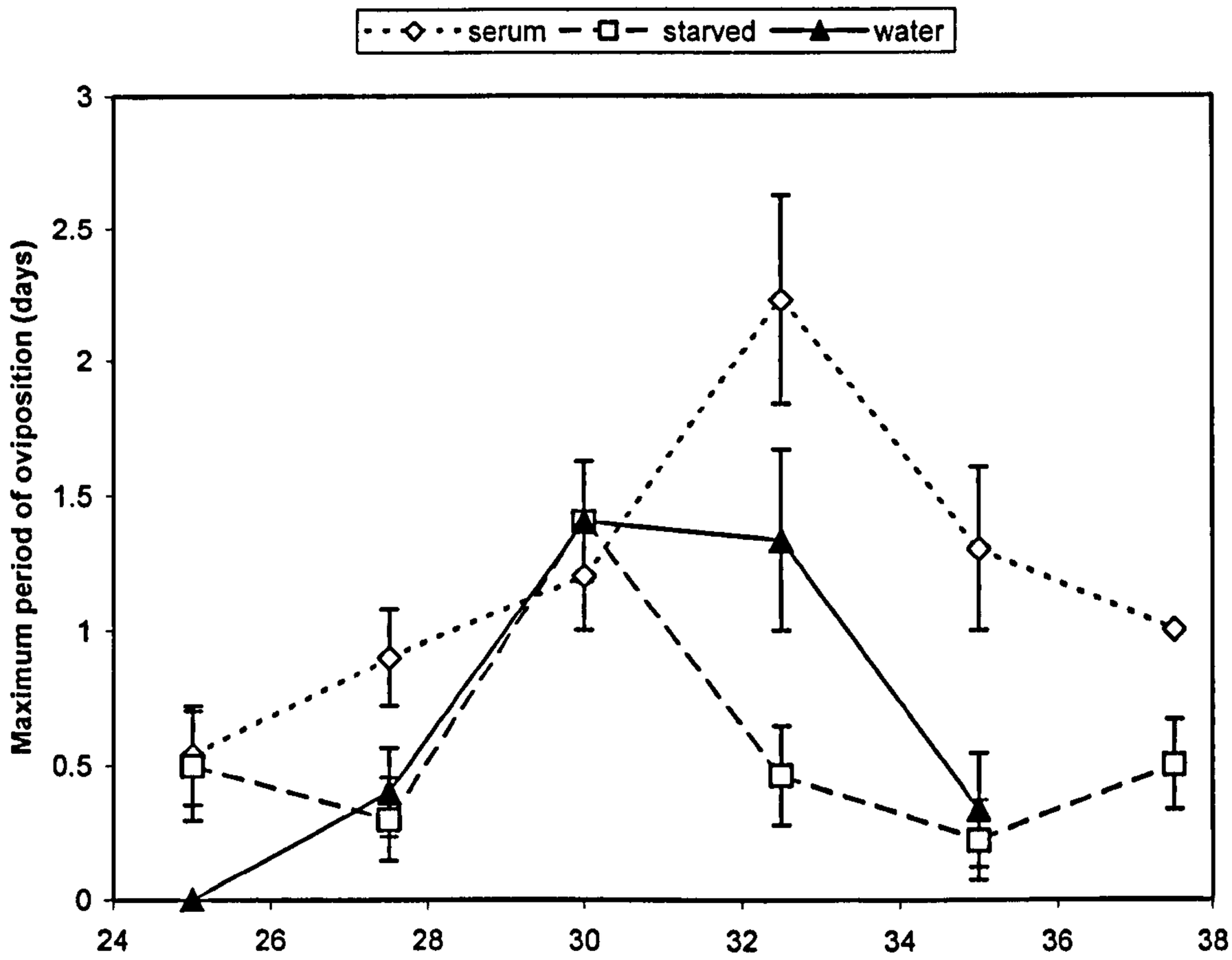


Figure 5.10 Percentage of *Psoroptes ovis* eggs that hatched when adult females and eggs were maintained on rabbit serum, distilled water or starved and incubated at a range of temperatures. Where hatch data is missing, this is due to the fact that no eggs were laid by adult female mites at some temperatures or, in a small number of cases, where eggs were lost by experimenter error.

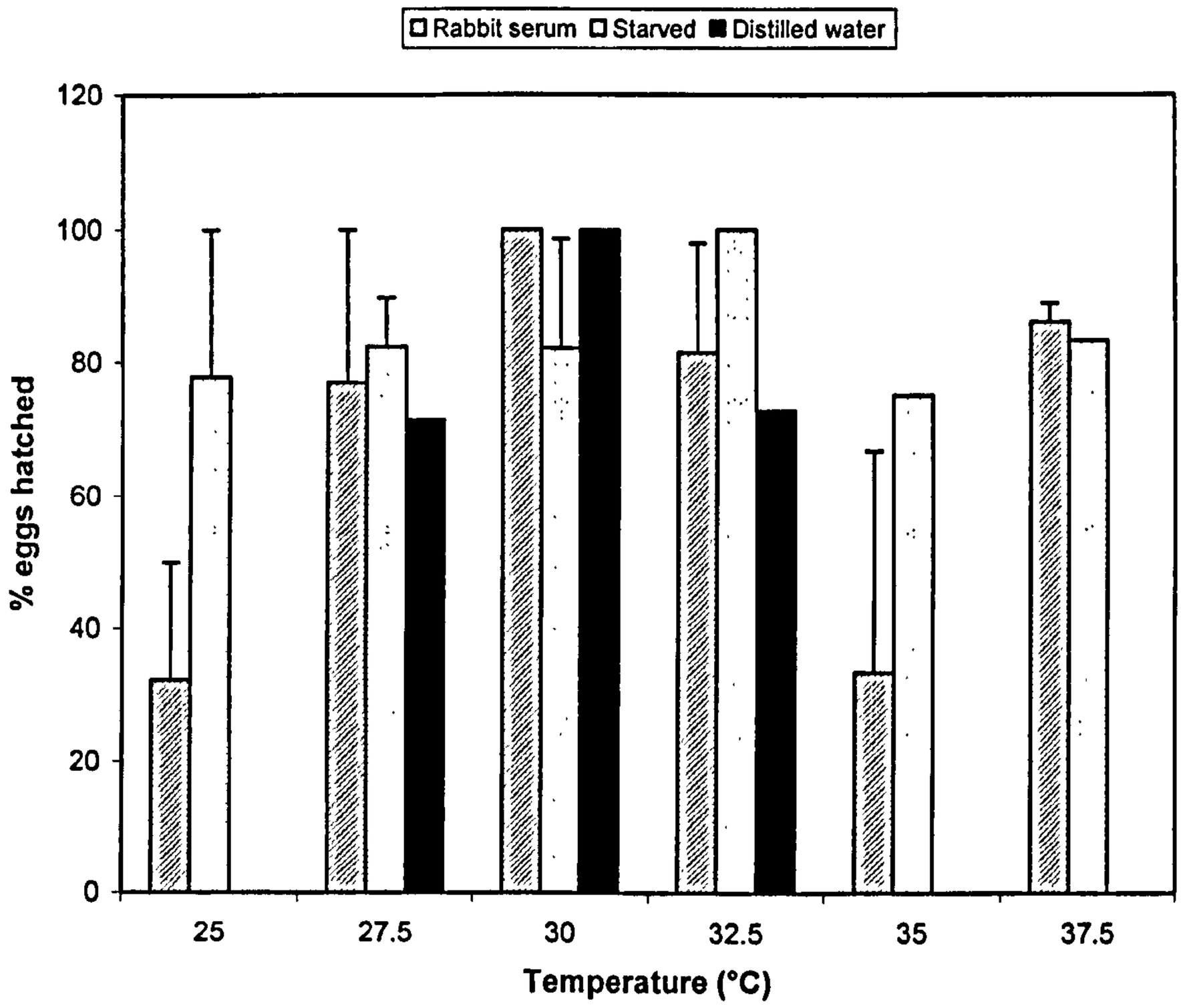


Figure 5.11 Mean time required for *Psoroptes ovis* eggs to hatch (±s.e.) when adult females and eggs were maintained (a) at a range of temperatures and (b) on rabbit serum or distilled water or starved.

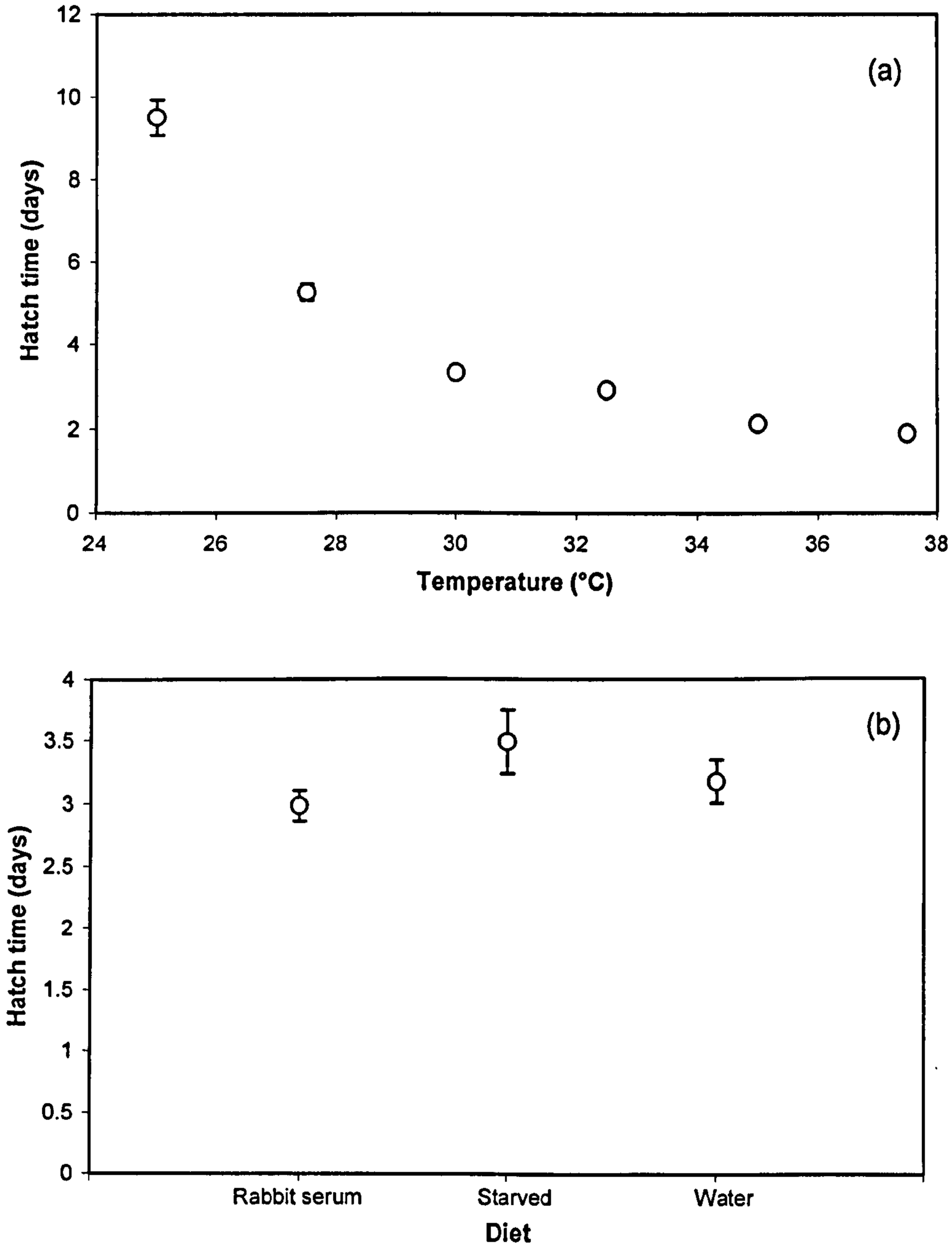


Figure 5.12 Mean time required for *Psoroptes ovis* eggs to hatch (±s.e.) when adult female mites and eggs were incubated at a range of temperatures and maintained on rabbit serum, starved or distilled water. Where hatch data is missing, this is due to the fact that no eggs were laid by adult female mites at some temperatures or, in a small number of cases, where eggs were lost by experimenter error. Points joined for clarity.

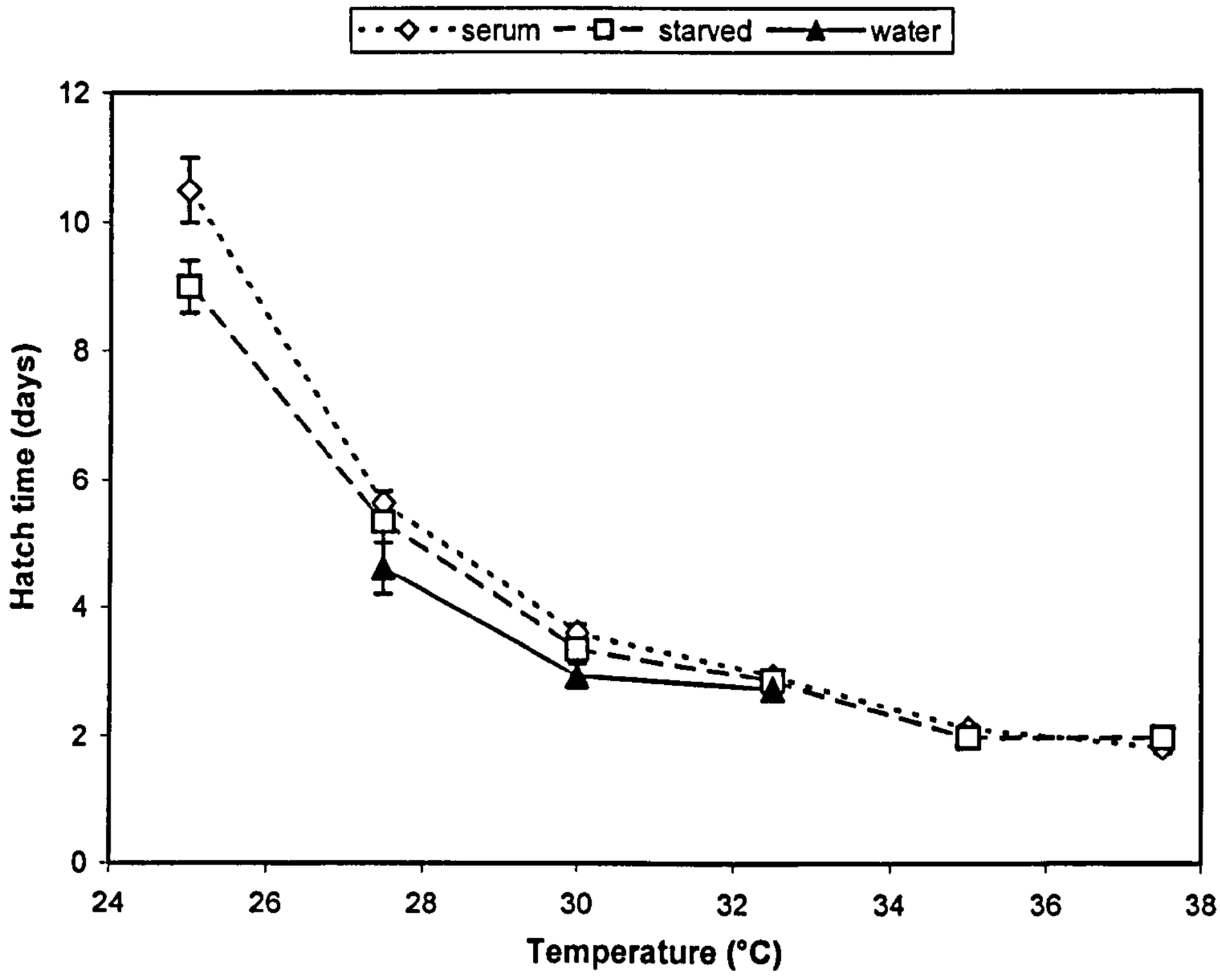
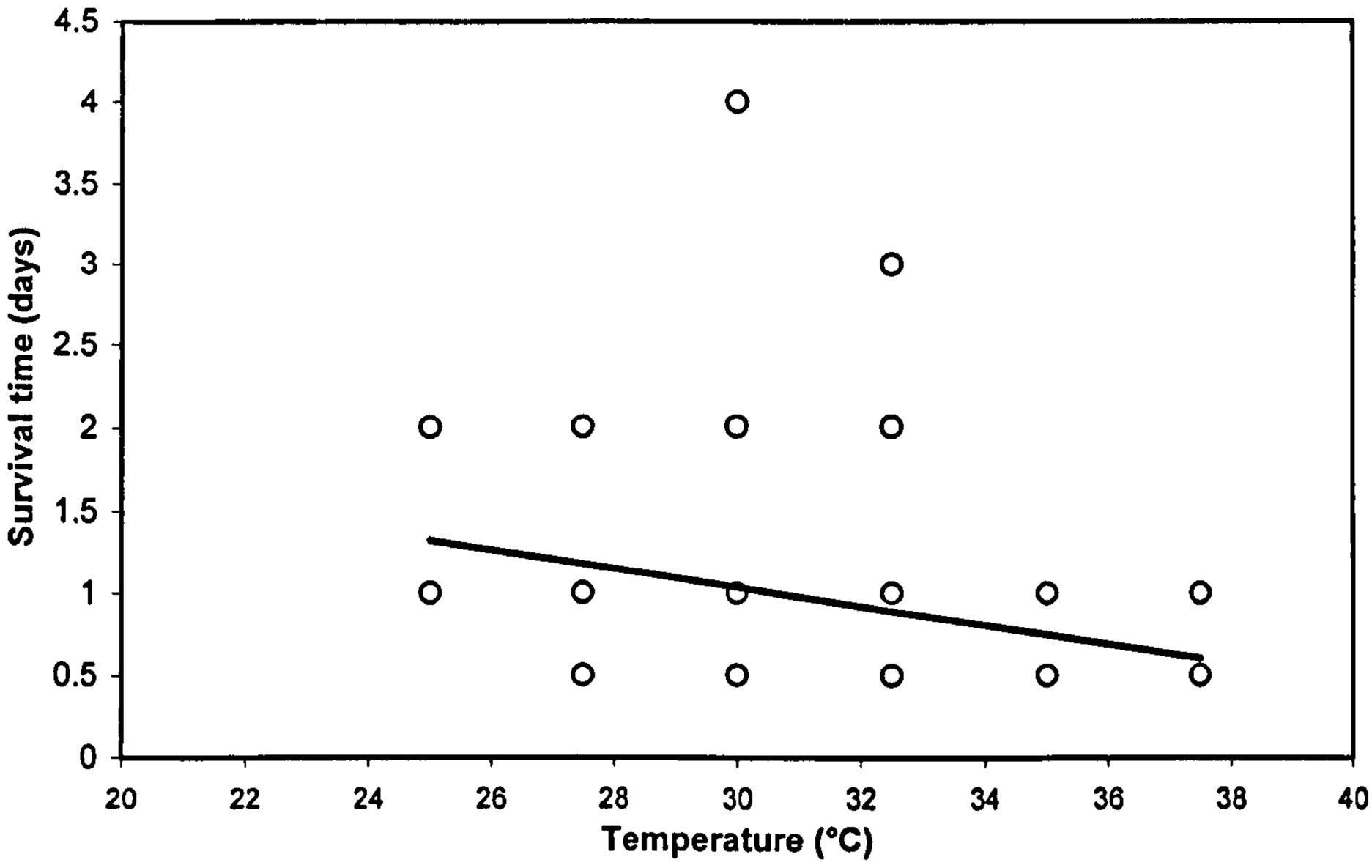


Figure 5.13 Survival time of *Psoroptes ovis* larvae maintained on rabbit serum, distilled water or starved (data pooled), with increasing temperature (fitted line: y=-0.057x + 2.76, r^2=13.3%).



5.4 Effect of diet

5.4.1 Materials and methods

For a more extensive study of the effects of diet on survival, chambers were incubated at 30°C but were provided with a range of different sources of nutrition. These included rabbit, sheep or cattle serum, keratinocyte culture medium (Sigma-Aldrich, Poole, UK), rabbit skin with either distilled water or rabbit serum, rabbit skin scrapings with either distilled water or rabbit serum, rabbit whole blood (in Alsevers, Harlan Sera-Lab, Loughborough, UK) and 50:50 mix of rabbit serum and distilled water. Again, there were two control treatments, mites were either given distilled water (control 1) or were starved by adding nothing to the chambers (control 2). Where mites were provided with rabbit skin, sections of approximately 25 x 25 mm were cut from the ears of dead European rabbits (killed during a shoot, decapitated and stored in a deep freeze until required). Where skin scrapings were provided, they were collected by scraping the inside surface of half a rabbit ear with a scalpel blade.

For adult female mites, a one-way ANOVA was used with LT_{50}, maximum survival or oviposition rate as the dependent variables and diet treatment as the factor. Similar ANOVAs were carried out for the egg and larval stages with percentage of eggs that hatched successfully, hatch time and survival time as the dependent variables, respectively. Tukey post-hoc tests were used to consider significant differences between diet treatment groups.

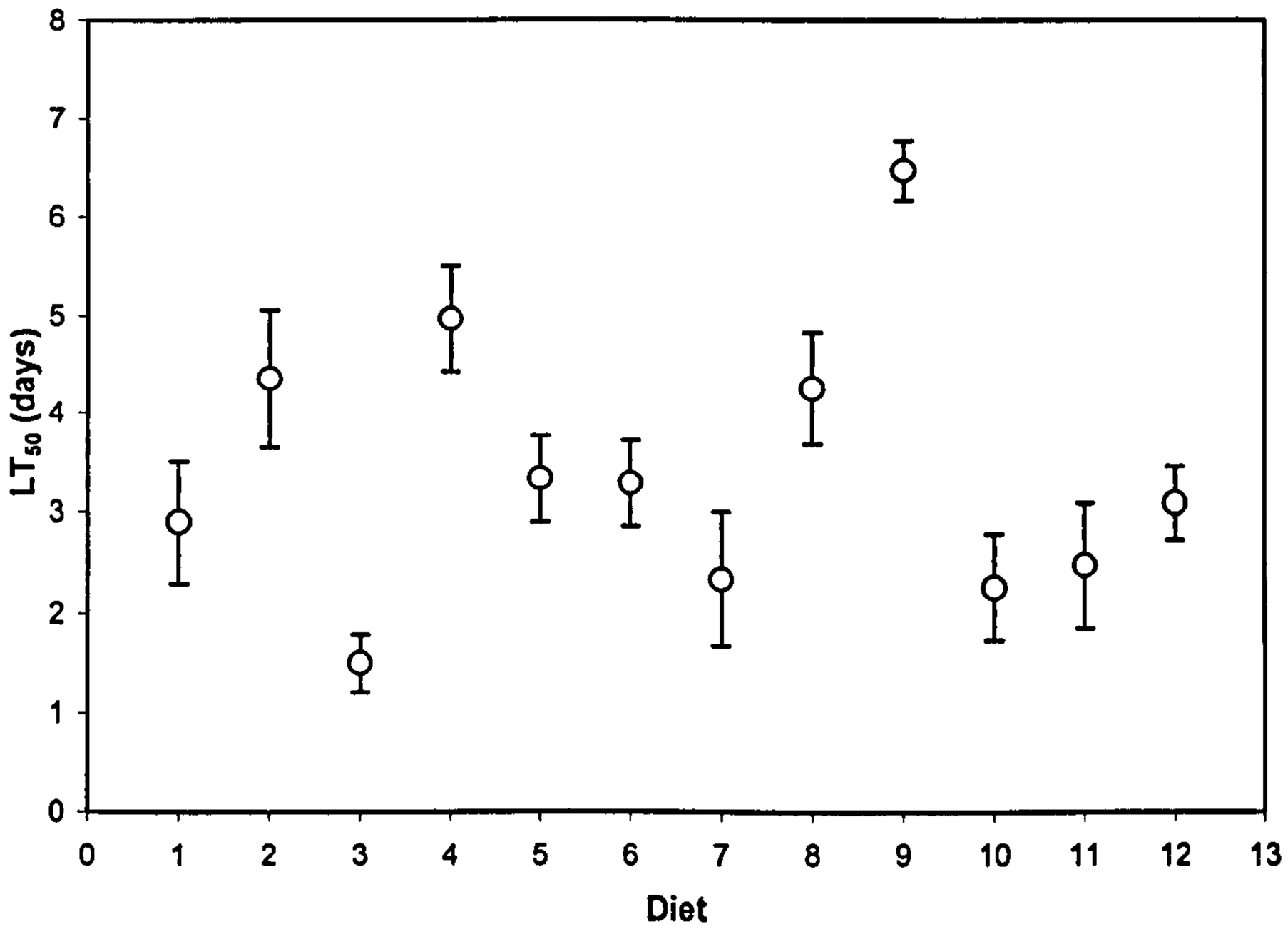
5.4.2 Results

The diet with which mites were provided had a significant effect on the LT_{50} ($F_{11,96}$=6.96, P<0.001; Fig. 5.14). The highest LT_{50} was observed in mites that were given calf serum and the lowest in mites that were given distilled water (control 1). Post-hoc tests revealed very few significant consistent differences between treatment groups with most of the differences being found between mites given calf serum or distilled water and the other treatment groups.

Diet also had a significant effect on the maximum survival time of the adult female mites ($F_{11,96}$=4.54, P<0.001; Fig. 5.15). Mites given calf serum had the longest maximum survival and those given distilled water (control 1) had the shortest maximum survival time. Post-hoc tests revealed very little consistent grouping between treatment groups.

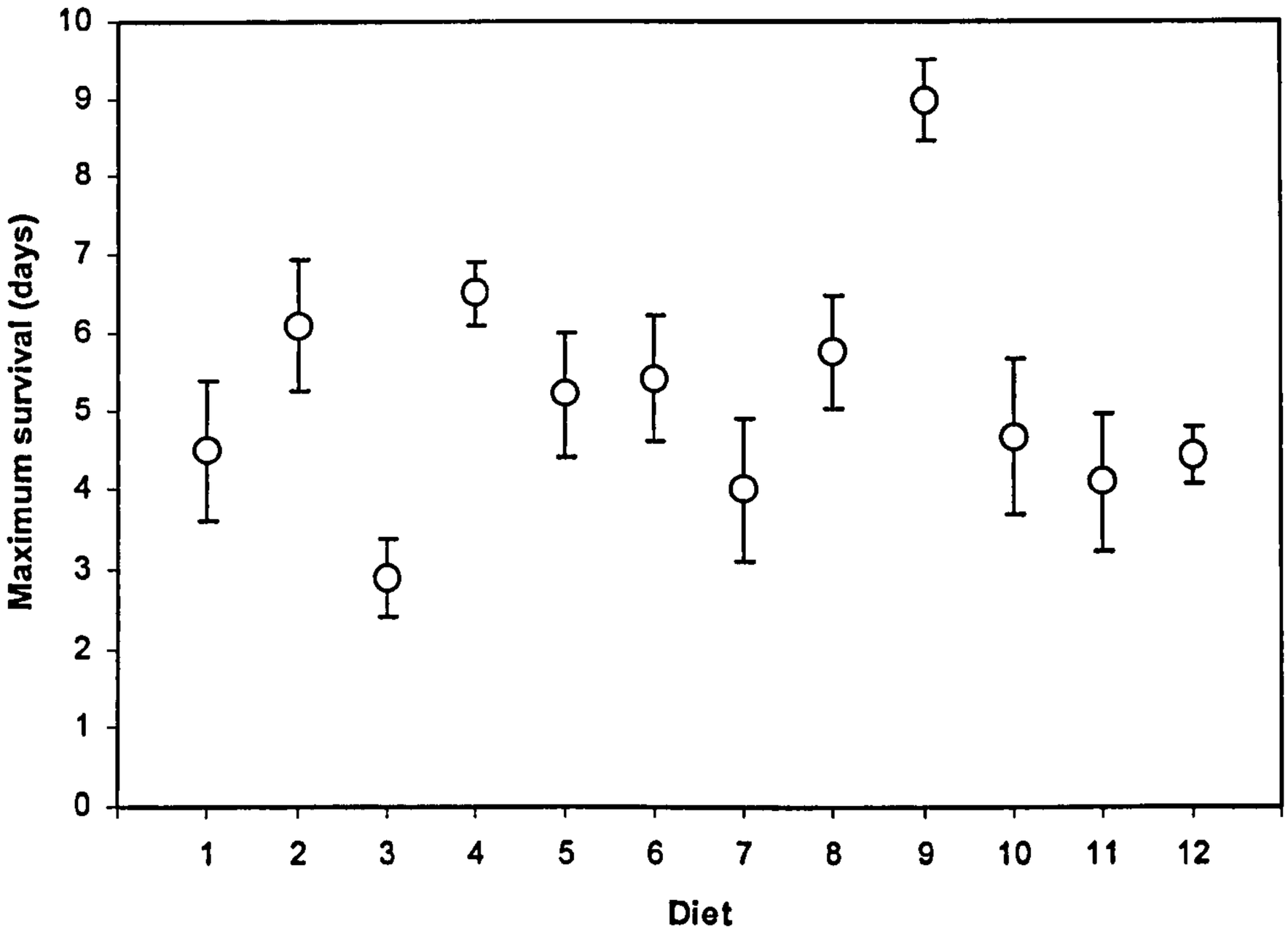
Oviposition rate varied significantly with diet ($F_{11,86}$=3.00, P=0.002; Fig. 5.16). The highest number of eggs were laid by adult females given rabbit blood, where almost 1 egg was laid for every female mite. The lowest number of eggs was laid by mites given distilled water where just 0.2 eggs were laid for every female in the first 24 hours. Very little consistent variation was observed in egg production between treatment groups.

Figure 5.14 Mean LT_{50} (±s.e.) for adult female *Psoroptes ovis* mites maintained on a range of diets.



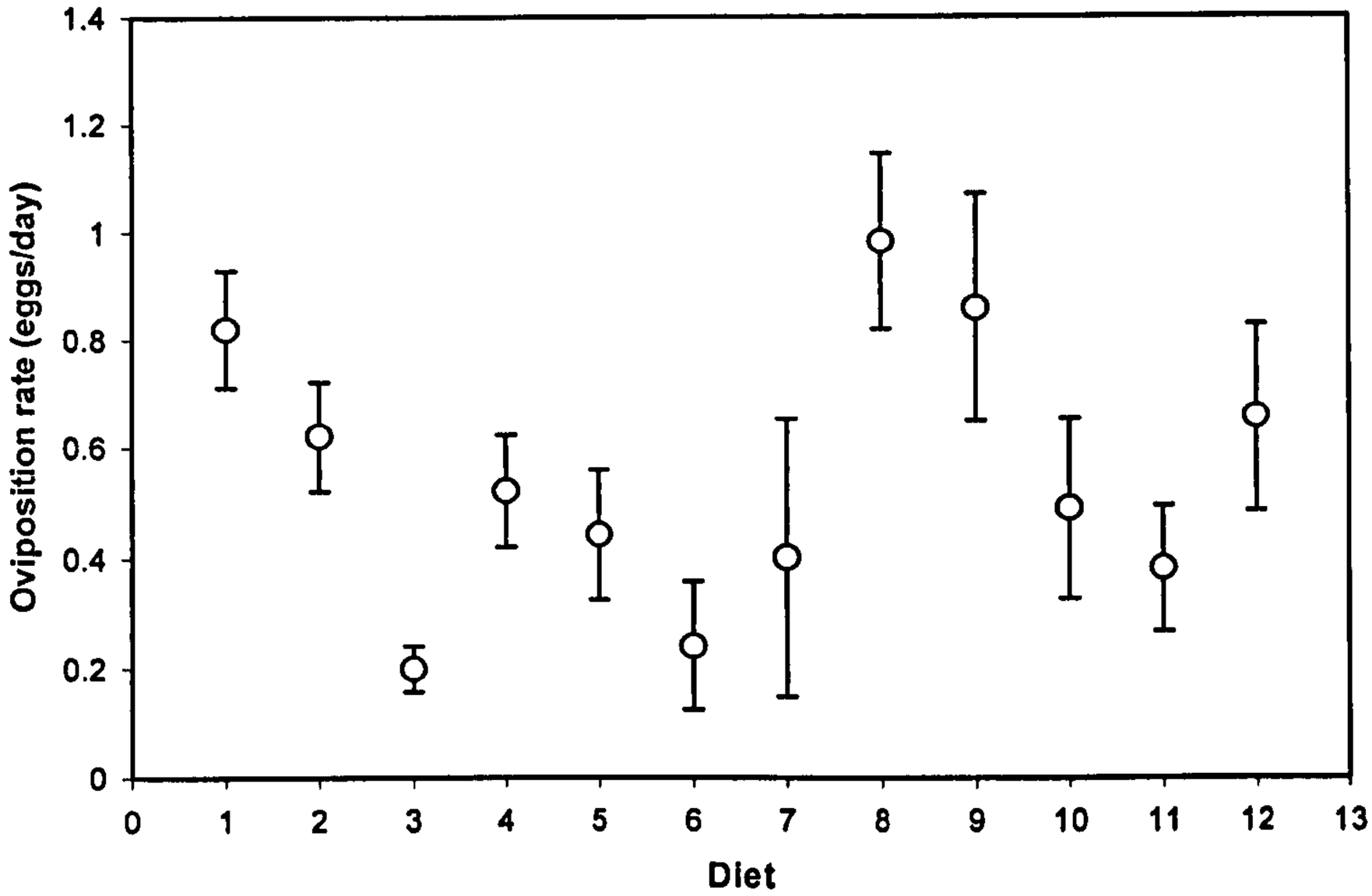
1 – Rabbit serum
2 – Starved
3 – Distilled water
4 – Rabbit skin + water
5 – Rabbit skin + serum
6 – Rabbit skin scrapings + water
7 – Rabbit skin scrapings + serum
8 – Rabbit Blood
9 – Calf serum
10 – Sheep serum
11 – Rabbit serum + water
12 – Keratinocyte medium

Figure 5.15 Mean maximum survival (±s.e.) of adult female *Psoroptes ovis* mites maintained on a range of diets.



1 – Rabbit serum
2 – Starved
3 – Distilled water
4 – Rabbit skin + water
5 – Rabbit skin + serum
6 – Rabbit skin scrapings + water
7 – Rabbit skin scrapings + serum
8 – Rabbit Blood
9 – Calf serum
10 – Sheep serum
11 – Rabbit serum + water
12 – Keratinocyte medium

Figure 5.16 Mean (±s.e.) number of eggs laid in the first 24h off-host by adult female *Psoroptes ovis* mites maintained on a range of diets.



1 – Rabbit serum
2 – Starved
3 – Distilled water
4 – Rabbit skin + water
5 – Rabbit skin + serum
6 – Rabbit skin scrapings + water
7 – Rabbit skin scrapings + serum
8 – Rabbit Blood
9 – Calf serum
10 – Sheep serum
11 – Rabbit serum + water
12 – Keratinocyte medium

At 30°C, the lowest percentage of eggs that hatched was 31% when adult females and eggs were maintained on rabbit skin with serum. The highest percentage of eggs that hatched successfully was 100%, and this occurred when adult females and eggs were maintained on rabbit serum or distilled water (Fig. 5.17). However, none of the differences in egg hatch rate observed were statistically significant.

The time taken for the eggs to hatch varied significantly with diet ($F_{11,224}$=154.72, P<0.001; Fig. 5.18). There were very few consistent differences between diet treatment groups, however, eggs maintained on calf serum took significantly longer to hatch than eggs from all other treatments except those maintained on rabbit skin with serum or rabbit blood.

Survival time of the larval stage of the mites was significantly affected by the diet treatment under which they were maintained ($F_{11,222}$=10.25, P<0.001). Post-hoc tests showed that there were no significant differences between treatment groups apart from larvae maintained on calf serum which survived significantly longer than all but one of the other treatment groups (Fig. 5.19).

Figure 5.17 Percentage of *Psoroptes ovis* eggs that hatched when adult females and eggs were maintained on a range of diets.

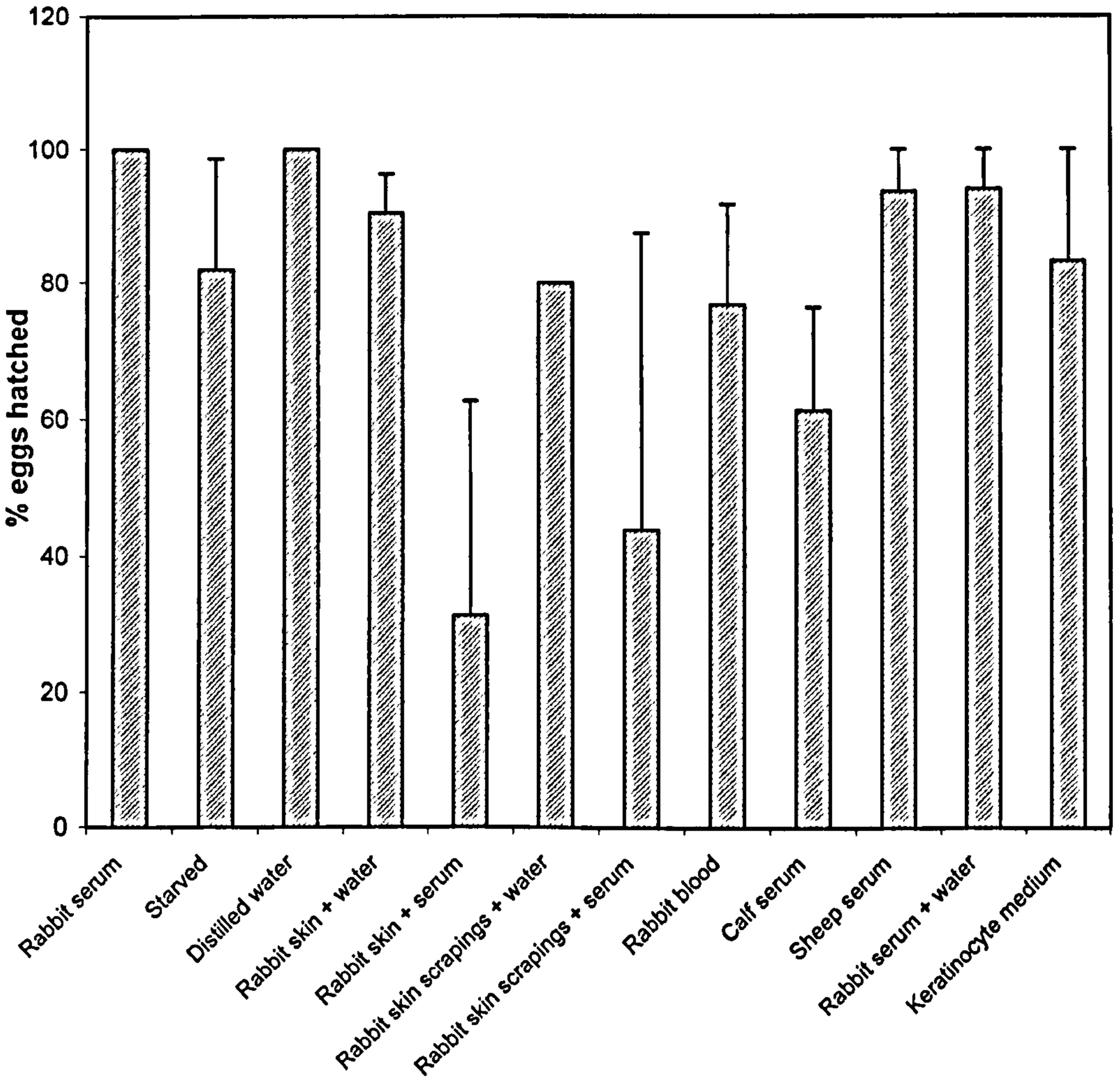
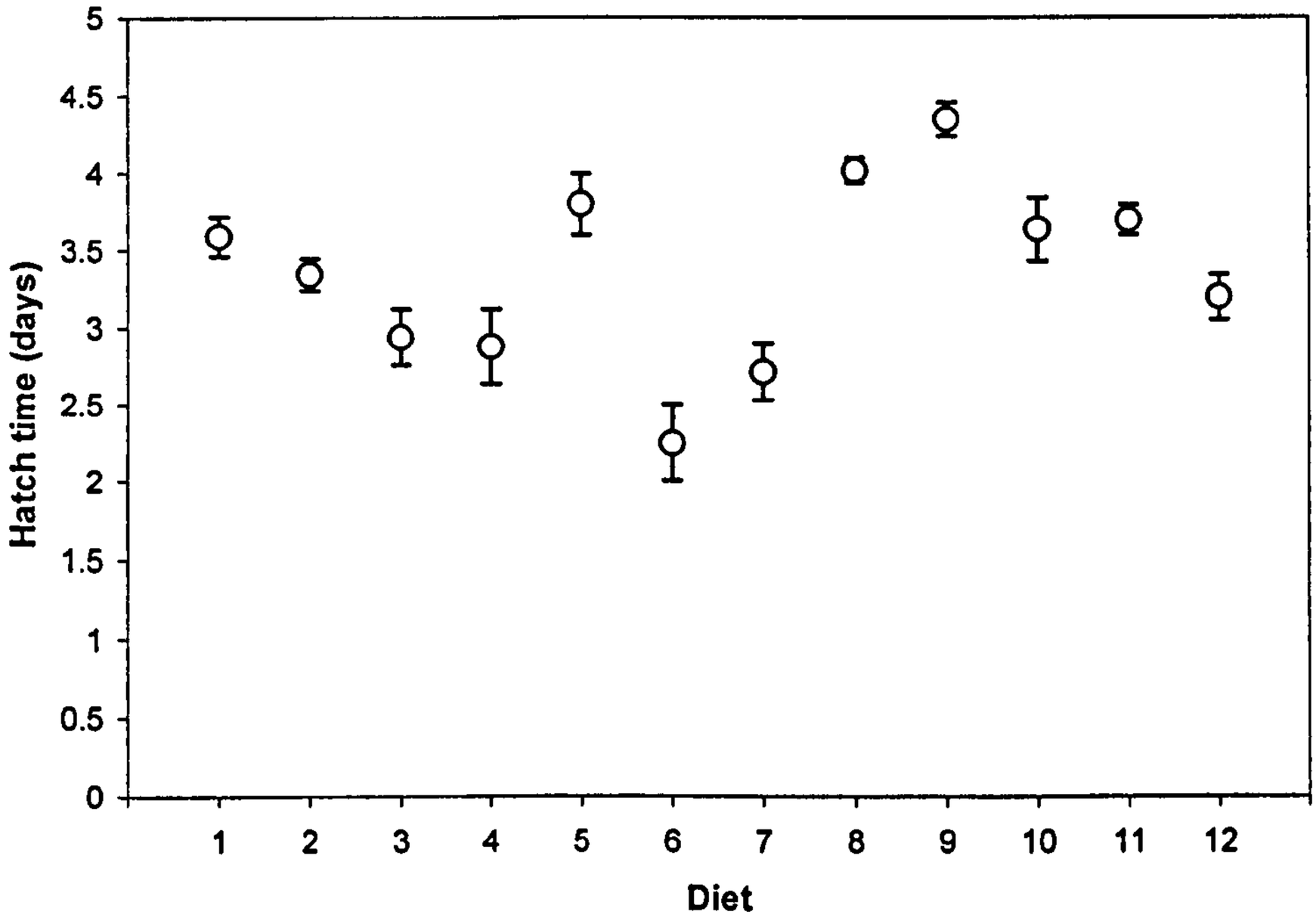
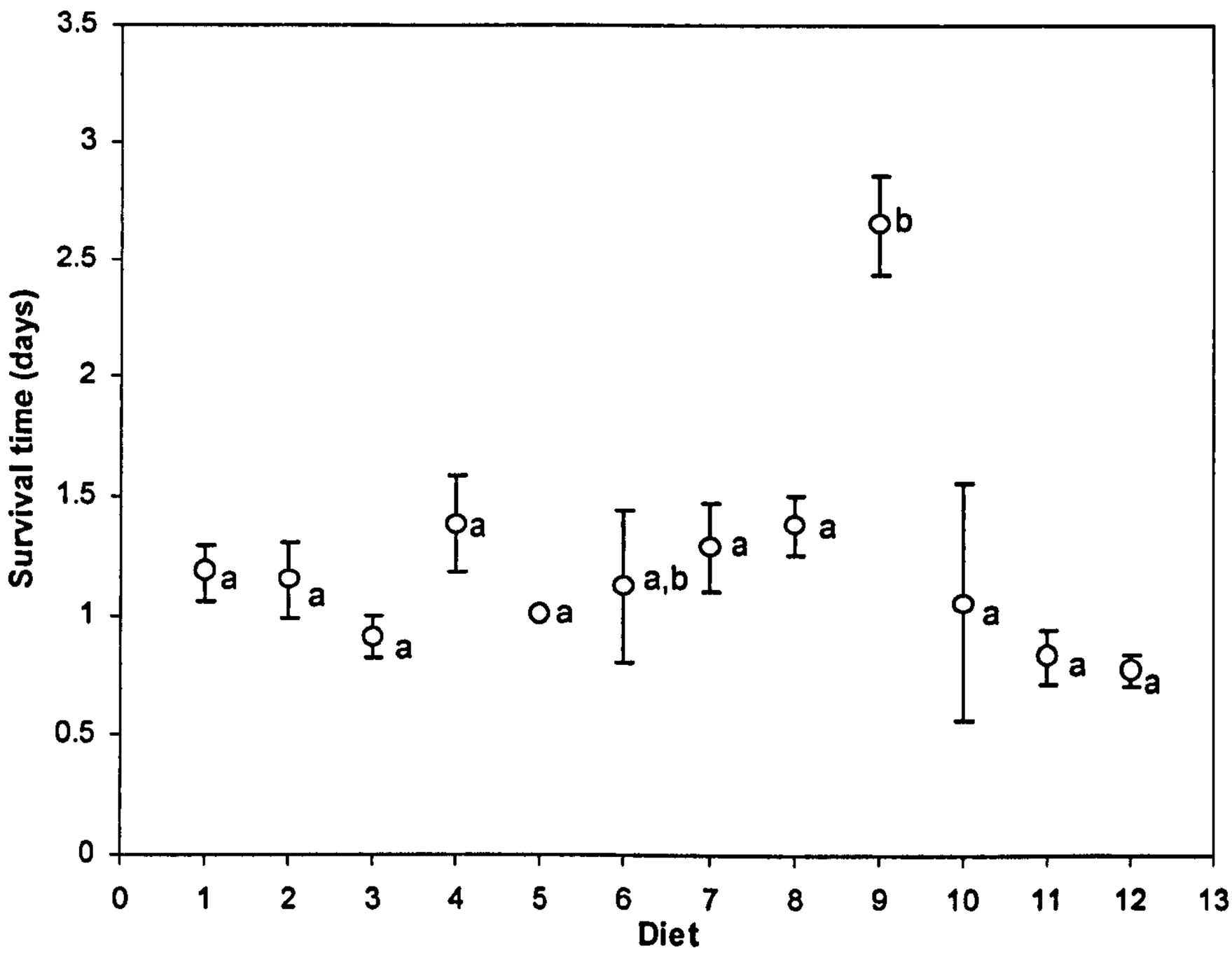


Figure 5.18 Mean time required for *Psoroptes ovis* eggs to hatch (±s.e.) when adult females and eggs were maintained on a range of diets.



1 – Rabbit serum
2 – Starved
3 – Distilled water
4 – Rabbit skin + water
5 – Rabbit skin + serum
6 – Rabbit skin scrapings + water
7 – Rabbit skin scrapings + serum
8 – Rabbit Blood
9 – Calf serum
10 – Sheep serum
11 – Rabbit serum + water
12 – Keratinocyte medium

Figure 5.19 Mean survival time (±s.e.) of *Psoroptes ovis* larvae maintained on a range of diets. Letters indicate data points between which there is no statistically significant difference.



5.5 *In vitro* feeding

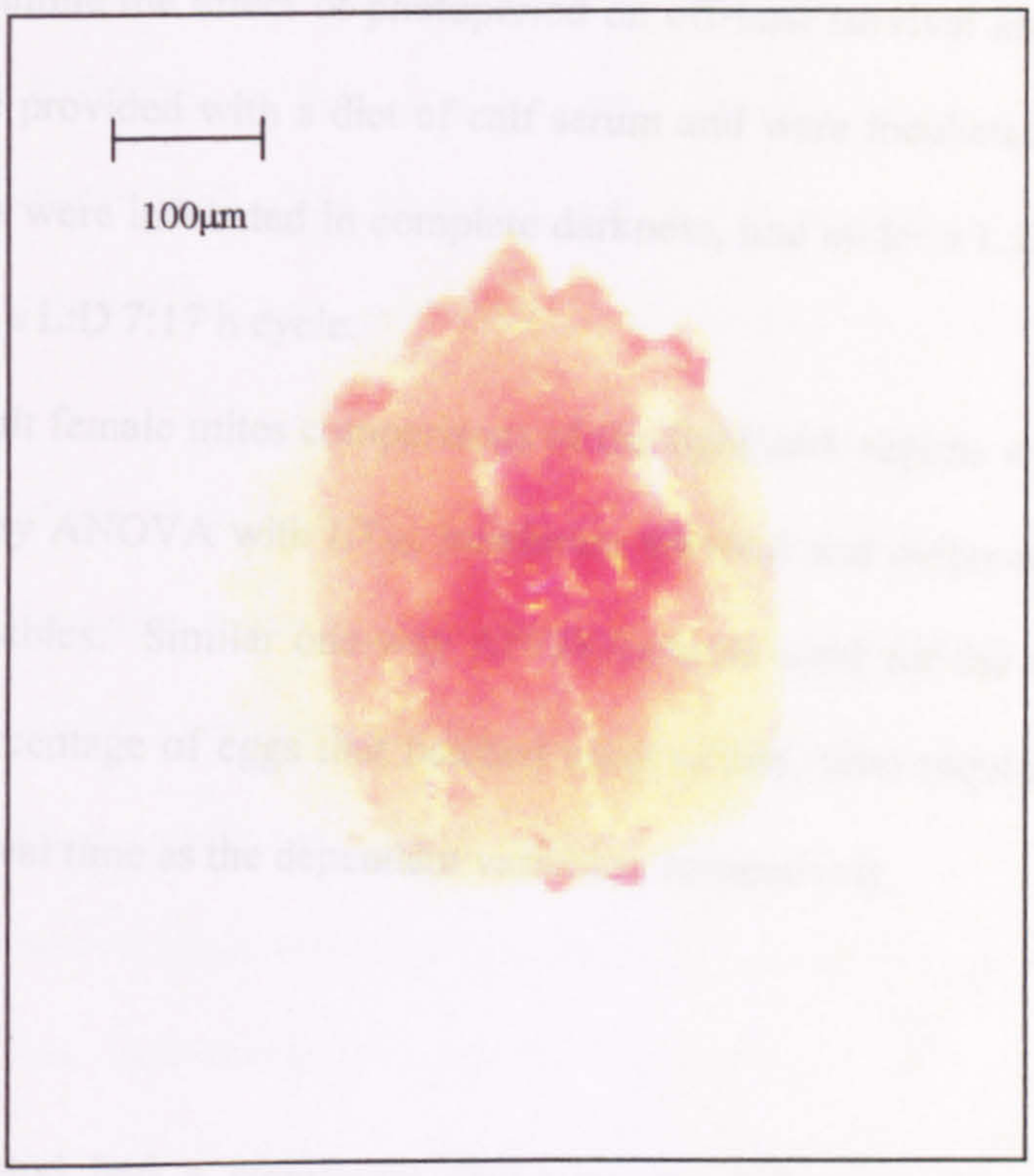
5.5.1 Materials and methods

To examine whether mites were indeed feeding on the diet they were provided with in the previous experiments, five groups of five adult female mites were given 200µl of calf serum (Sigma-Aldrich, Poole, UK) containing a red dye (Food colouring, Supercook, Leeds, UK) added at a ratio of 1 part dye to 5 parts serum. Chambers were incubated at 30°C for 24 hours after which the number of mites that had fed was counted. Mites that had fed were identified by the presence of a red patch of colour in the centre of their body.

5.5.2 Results

Successful *in vitro* feeding was observed, with 24% of mites ingesting the calf serum within 24 hours off-host (Fig. 5.20).

Figure 5.20 *Psoroptes* mite following ingestion of liquid media containing a red food dye.



5.6 Effect of photoperiod

5.6.1 Materials and methods

To examine the effect of photoperiod on off-host survival and development, all mites were provided with a diet of calf serum and were incubated at 30°C. One group of mites were incubated in complete darkness, one under a L:D 12:12 h cycle and one under a L:D 7:17 h cycle.

For adult female mites comparisons of the light/dark regime were undertaken using a one-way ANOVA with LT_{50}, maximum survival and oviposition rate as the dependent variables. Similar one-way ANOVAs were used for the egg and larval stages with percentage of eggs that hatched successfully, time required for eggs to hatch and survival time as the dependent variables, respectively.

5.6.2 Results

Photoperiod had a significant effect on the LT_{50} of adult female mites ($F_{2,27}$=12.08, P<0.001). Mites incubated under a L:D 7:17 regime had a significantly shorter LT_{50} than those incubated under a L:D 12:12 regime or in complete darkness (Fig. 5.21).

Maximum survival was also significantly affected, but to a lesser extent than LT_{50}, by light treatment ($F_{2,27}$=4.61, P=0.019). Mites maintained in complete darkness had a significantly longer maximum survival than those maintained under a L:D 7:17 regime but not than those maintained under a L:D 12:12 regime. Mites maintained under the two different photoperiods did not differ significantly in their maximum survival times (Fig. 5.22).

Light treatment had a significant effect on oviposition rate ($F_{2,27}$=5.51, P=0.01). Mites incubated in complete darkness or under a L:D 7:17 regime laid significantly more eggs than those incubated under a L:D 12:12 regime. The

oviposition rate did not differ significantly between mites incubated in complete darkness or under a short photoperiod (Fig. 5.23).

Light treatment had a significant effect on the time required for eggs to hatch ($F_{2,126}$=29.08, P<0.001). Tukey post-hoc tests showed that hatch time differed significantly between each of the treatment groups with eggs taking longest to hatch when kept in complete darkness and hatching most quickly under a long photoperiod (Fig. 5.24).

The presence of a photoperiod had no significant effect on the egg hatch success rate. However, a greater egg hatch success rate was observed when eggs were incubated under a photoperiod (Fig. 5.25) with eggs maintained under a short photoperiod giving a slightly better hatch rate.

Larval survival time was significantly affected by the light regime under which mites were maintained ($F_{2,124}$=9.87, P<0.001). Larvae survived for a significantly longer period of time when kept in constant darkness than when kept under a photoperiod (Fig. 5.26).

Figure 5.21 Mean LT_{50} (±s.e.) for adult female *Psoroptes ovis* mites maintained on calf serum at 30°C whilst incubated under constant darkness or one of two photoperiods. Letters indicate groups between which there is no statistically significant difference.

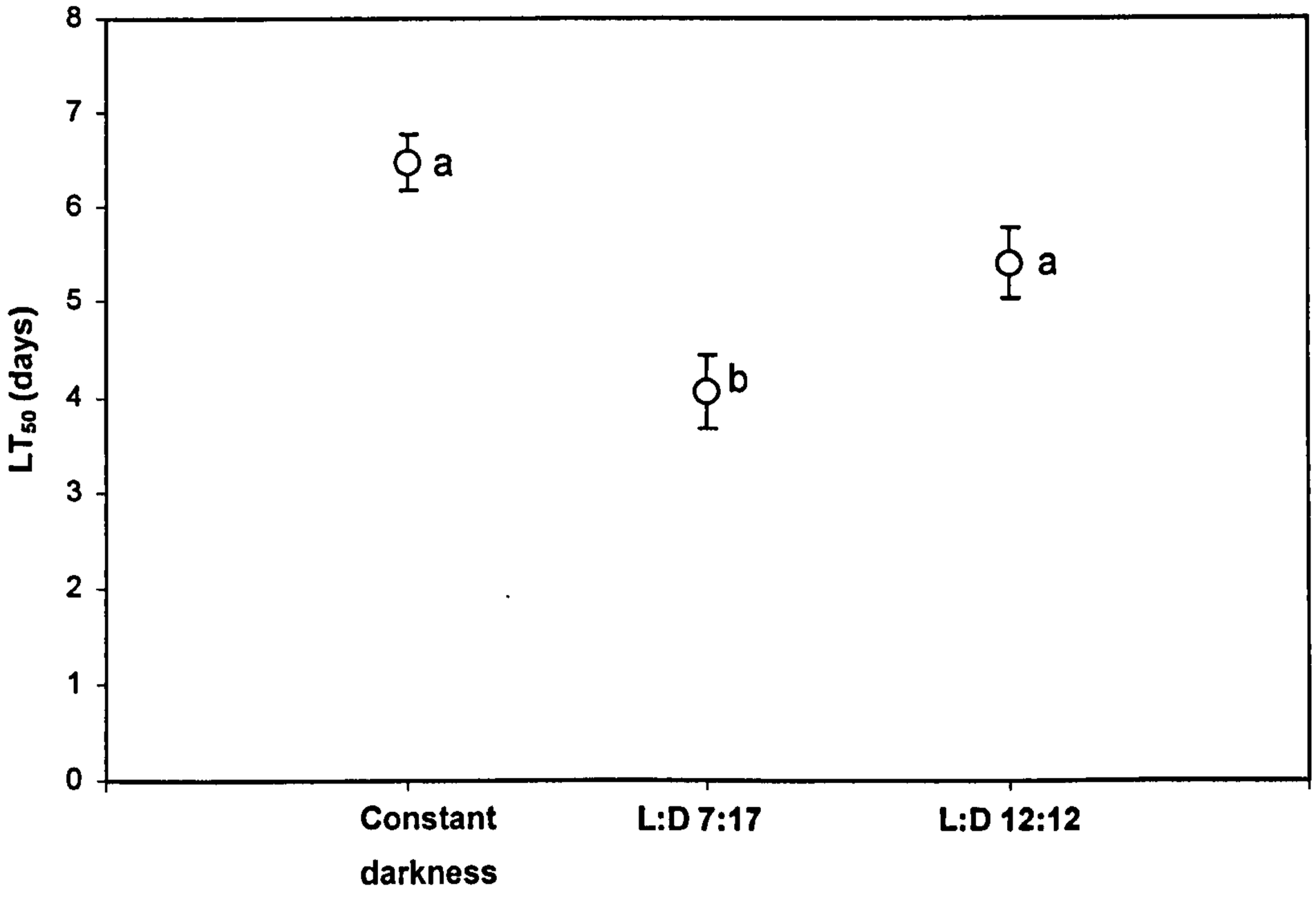


Figure 5.22 Mean maximum survival (±s.e.) for adult female *Psoroptes ovis* mites maintained on calf serum at 30°C whilst incubated under constant darkness or one of two photoperiods. Letters indicate points between which there is no statistically significant difference.

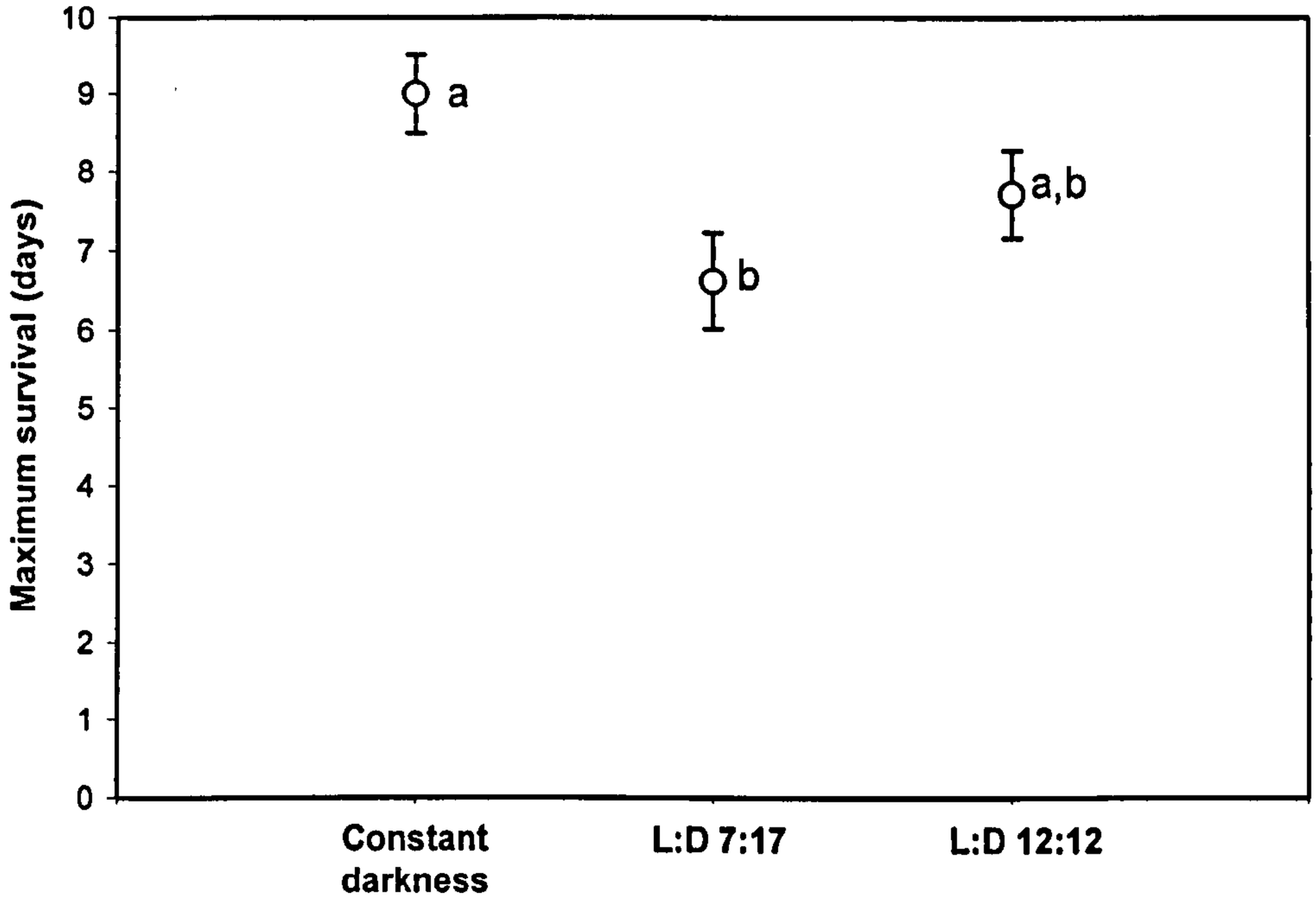


Figure 5.23 Mean oviposition rate (±s.e.) of adult female *Psoroptes ovis* mites maintained on calf serum whilst incubated under constant darkness or one of two photoperiods. Letters indicate groups between which there is no statistically significant difference.

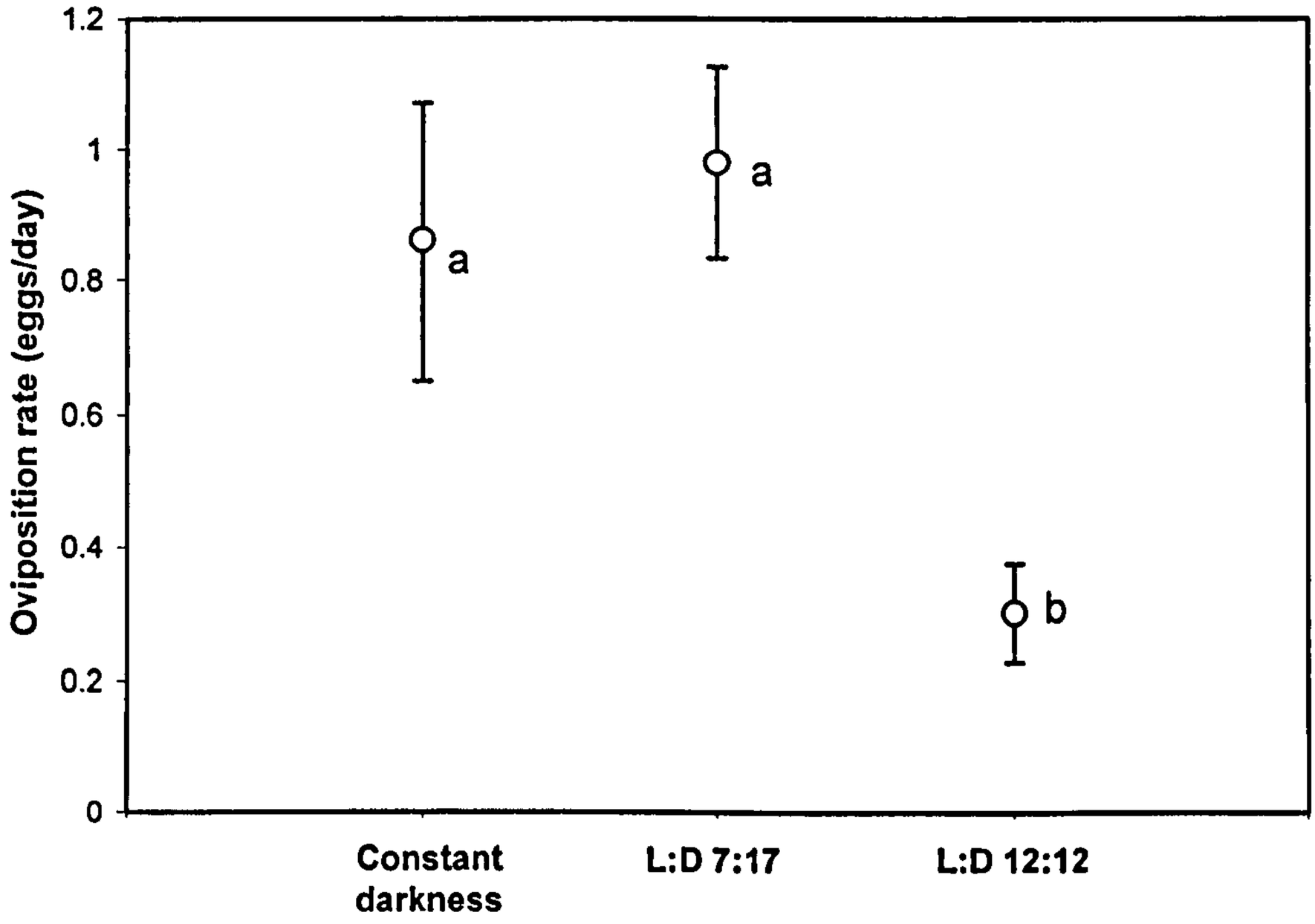


Figure 5.24 Mean time required for *Psoroptes ovis* eggs to hatch (±s.e.) when incubated at 30°C under constant darkness or under one of two photoperiods when adult females and eggs were maintained on calf serum. Letters indicate groups between which there is no statistically significant difference.

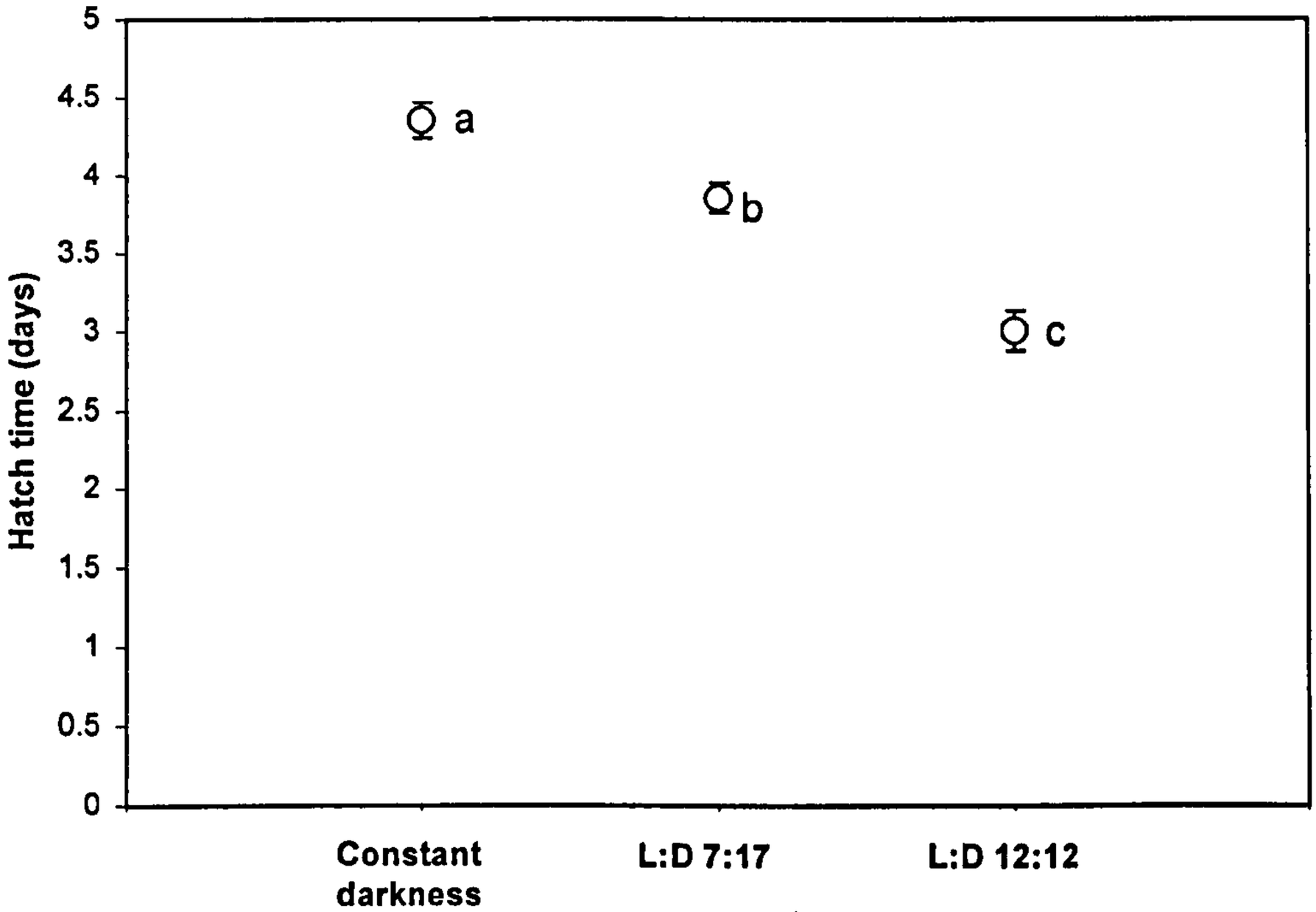


Figure 5.25 Percentage of *Psoroptes ovis* eggs that hatched when adult females and eggs were maintained on calf serum at 30°C when kept in constant darkness or under one of two photoperiods.

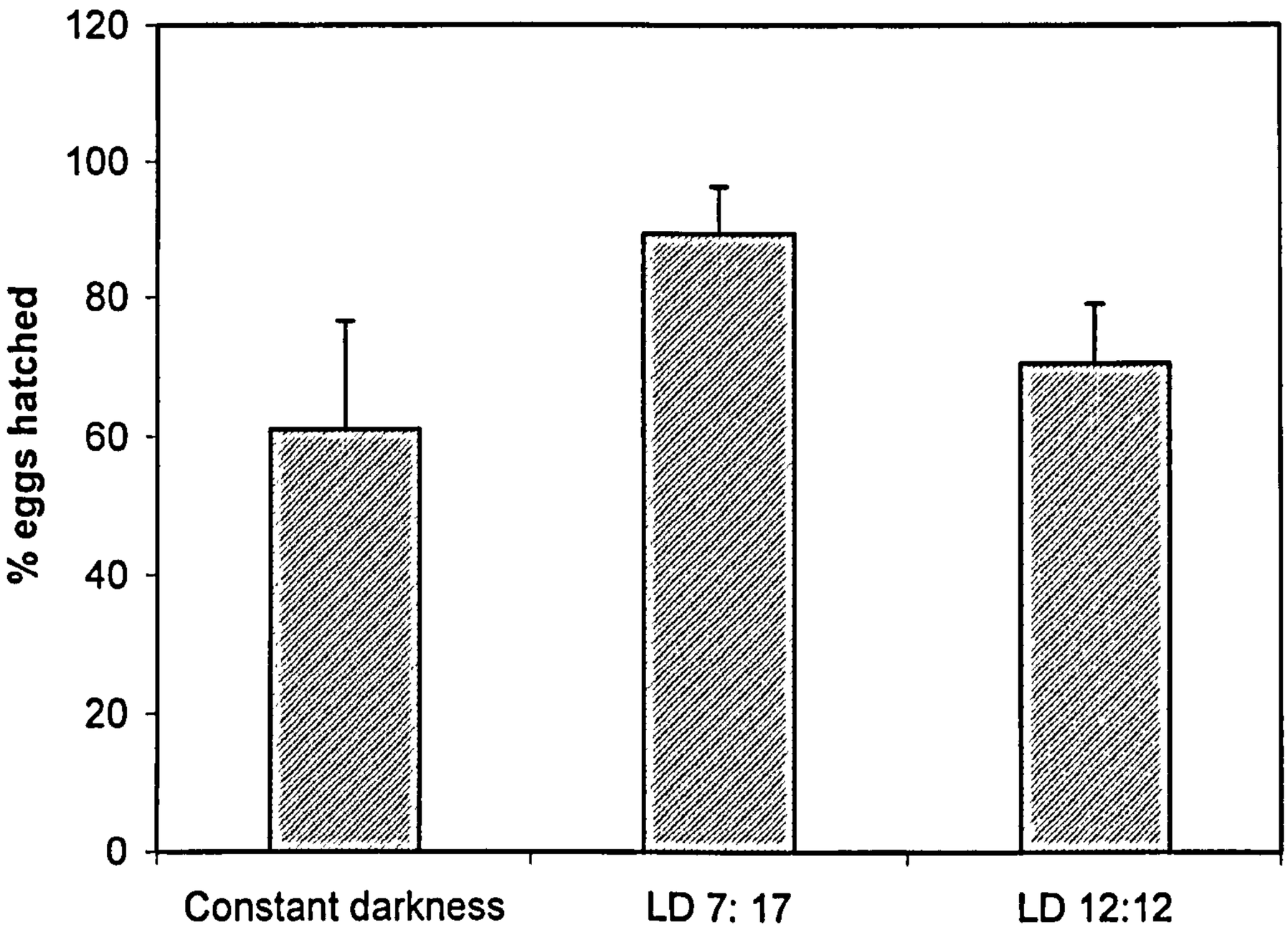
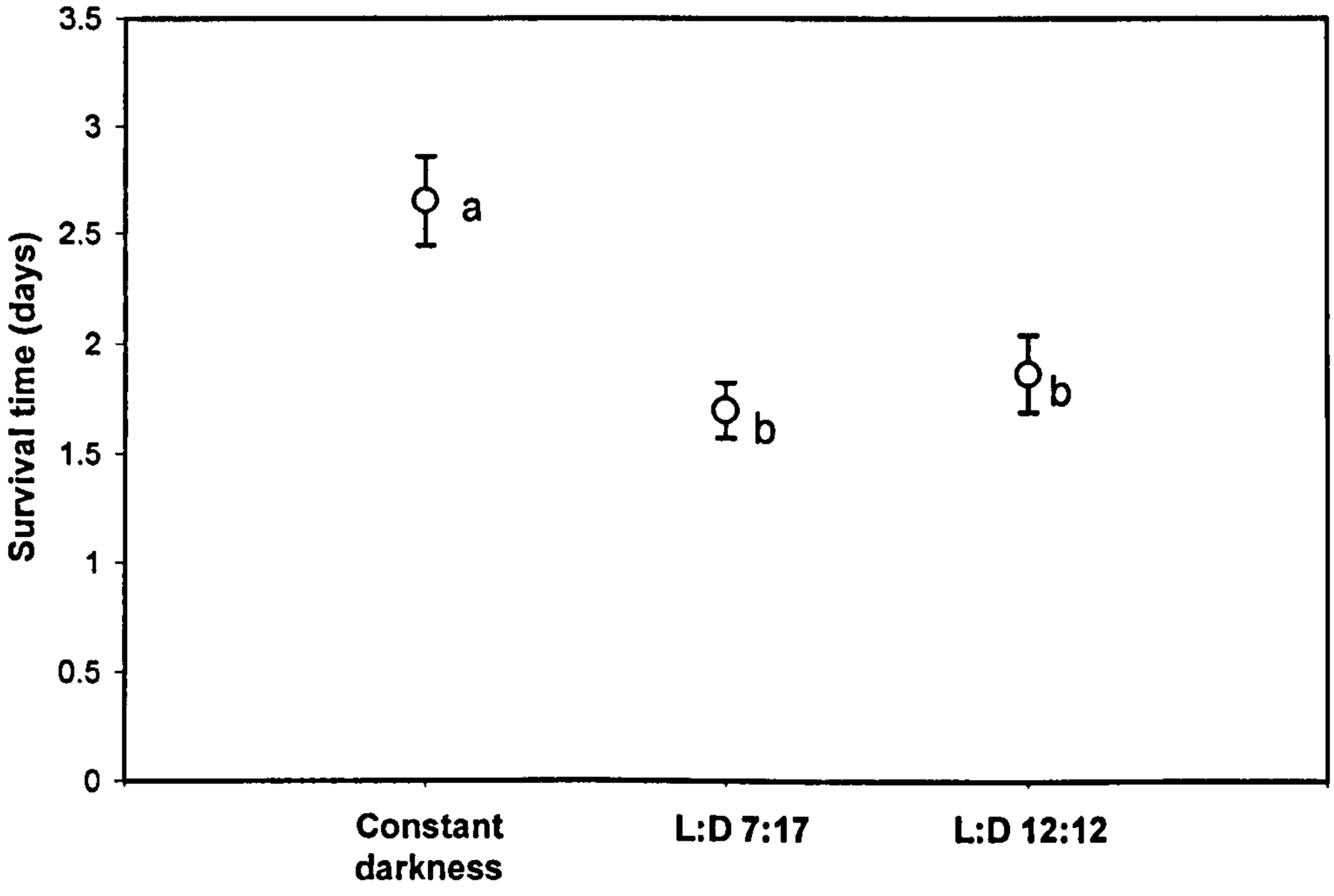


Figure 5.26 Mean survival time (±s.e.) of *Psoroptes ovis* larvae maintained on calf serum at 30°C when kept in constant darkness or under one of two photoperiods. Letters indicate points between which there is no statistically significant difference.



5.7 Discussion

The survival of adult female *Psoroptes* mites off the host has been relatively well documented and it has been shown that off-host survival is affected by temperature (Liebisch *et al.*, 1985; Smith *et al., 1999, Meintjies et al., 2002c). These* previous studies found that there was a decrease in the longevity of adult females with increasing temperature; at 25°C survival was as short as 3 days (Meintjies *et al.*, 2002c). A similar negative relationship between longevity and temperature has been found in other mite species such as *Otodectes cynotis* (Hering) (Otranto *et al.*, 2004) and *Euseius finlandicus* Oudemans (Broufas and Koveos, 2001) and in ticks, for example, *Dermacentor reticulatus* (Fabricius) (Zahler and Gothe, 1995). In the present study, a decrease in LT_{50} and maximum survival time with increasing temperature was observed between 25 and 27.5°C and at temperatures above 32.5°C. However, at temperatures of between 27.5 and 32.5°C, LT_{50} and maximum survival time increased slightly with increasing temperature and reached their maximum values between these temperatures. Mathieson (1995) looked at survival of adult female *Psoroptes* mites at a temperature range of 20-37°C and found survival time to be greatest at 33°C.

Meintjes *et al.* (2002c) found that *Psoroptes* larvae survived for greater periods of time when maintained at lower temperatures. A significant negative relationship between larval survival time and temperature was also found here. It is likely that the larval development rate was greater at higher temperatures but some constraint prevented the moulting of larvae into protonymphs. It has been suggested that the average duration of the larval stage is 2.2 days (Downing, 1936b; Wall *et al.*, 1999) and in this study, larval survival time lasted from 0.5-4 days, therefore, some of the larvae had survived long enough to moult.

It has been suggested that the rate of oviposition in *Psoroptes* mites is reduced at lower temperatures (Mathieson, 1995) and reduced oviposition has been observed at both high and low temperatures in *Dermanyssus gallinae* (De Geer) (Nordenfors *et al.*, 1999). In the present study, oviposition rate by adult females was significantly affected by temperature. The number of eggs laid was relatively low at low temperatures but greater at increased temperatures, with maximum oviposition rate being reached at between 30 and 32.5°C. However, at no time did oviposition rate reach levels observed on the host (Downing, 1936b; Wall *et al.*, 1999) and females were very rarely seen to lay eggs when they had been removed from the host for more than two days, as observed by Stockman and Berry (1913) and Shilston (1915).

In hatching experiments carried out by Meintjes *et al.* (2002c), egg hatching success varied between 7.14% at 10°C and 35.71% at 25°C. Egg hatch success in the present study was comparatively very good, with a 100% success rate achieved in some cases. Although the percentage of eggs that hatched successfully appeared to be lower at reduced temperatures, there was no significant effect of temperature.

The time required for *Psoroptes* eggs to hatch has been observed to decrease with increasing temperatures (Meintjies *et al.*, 2002c) and a similar trend was also observed in *E. finlandicus* (Broufas and Koveos, 2001). In the present study, the time taken for eggs to hatch was found to be significantly affected by temperature with hatch times decreasing at higher temperatures. The hatch times for eggs observed here, were not outside the estimates of hatch times on host of 2-4 days (Stockman and Berry, 1913; Shilston, 1915; Downing, 1936b; Wall *et al.*, 1999) suggesting that off-host rearing of *Psoroptes* is not constrained by the egg hatch success or rate of development.

Smith *et al.* (1999) found that the maintenance of *Psoroptes* mites on rabbit serum gave increased survival compared to mites maintained on distilled water. In the present study, diet was also observed to have a significant effect on LT_{50} and maximum survival time of adult female *Psoroptes* mites, suggesting that mites are feeding or gaining energy resources from particular diets in some way. Mites maintained on calf serum were observed to ingest the medium with which they were provided. When a range of potential diets were tested, calf serum was found to maximise survival. Population sizes of mites infesting cattle are generally larger than in mites infesting sheep and are able to inhabit the whole of the lesion where they are seen to move to the edge when infesting sheep. (Kirkwood, 1986). These observations may suggest that *Psoroptes* mites survive better on cattle, making calf serum a more suitable diet than serum from other hosts. A significant interaction between diet and temperature was observed. This may result in different diets having different effects on longevity when mites are maintained at different temperatures. Therefore, although calf serum appears to be the most suitable diet at 30°C, a different diet may be more beneficial to longevity at a different temperature.

Diet had a very small but in some cases, significant effect on the survival time of the larval stage of *Psoroptes*. Where the effect of temperature was examined, whether the larvae were maintained on rabbit serum, distilled water or starved had no significant effect on their survival time. When a range of potential diets were tested whilst larvae were maintained at 30°C, only calf serum and rabbit skin scrapings with water enhanced survival times significantly.

Stockman and Berry (1913) stated that it was unusual for a *Psoroptes* adult female to lay more than one egg following removal from the host unless it was returned to the host to feed. In the present study, diet had a significant effect on the rate of oviposition of adult female *Psoroptes* mites, with rabbit blood being the best

medium to produce the maximum egg output when maintained at 30°C. The maximum egg output achieved was 0.98 eggs per female in 24 h which is still considerably lower than the on-host estimate of 2.9 eggs per female per day (Downing, 1936b; Wall *et al.*, 1999). However, since oviposition ceased soon after removal from the host, this suggests that the diets provided here are unlikely to have provided sufficient nutrition to the mites.

The time taken for eggs to hatch was significantly affected by medium added to the chamber, with eggs to which calf serum was added taking the longest to time hatch and those placed on rabbit skin scrapings with distilled water hatching in the shortest time. This is a surprising result since clearly eggs are not a feeding stage. However, since eggs are maintained on a medium that the adult female mite that produced the egg was also maintained on, it may be that particular diets enabled the female mite to invest more energy into an egg.

There is evidence to suggest that serum and exudates from sheep may have a detrimental effect on mite survival (Sinclair and Filan, 1991). However, a positive feeding response to bovine serum has been observed in *Psoroptes* mites reared on cattle (DeLoach, 1984) and mites have been shown to ingest red blood cells (DeLoach and Wright, 1981). Mathieson (1995) found that mites do ingest components of sheep serum and it has also been suggested that mites feed on epidermal lipid (Sinclair and Filan, 1989). DeLoach (1984) and Mathieson (1995) have observed mites feeding on different substances but have failed to enhance the off-host survival of un-fed mites. These studies show that serum, blood and skin components are likely to be necessary nutritional requirements for *Psoroptes* mites, but perhaps other components are also required to maximise survival and development. Finding the most suitable diet for *Psoroptes* mites is of considerable importance, not only in the development of an *in vitro* rearing system but also in

methods of control. Any novel systemic control approaches such as acaricides or vaccines may only be successful if the mites do feed on host-derived substances.

It has been demonstrated that *Psoroptes* mites are able to respond to light (Chapter 4), so it is therefore possible that light-sensitive circadian clocks may regulate the timing of various aspects of their life cycle, although no studies have examined this previously. Circadian clocks are known to control the timing of events including behavioural, physiological and biochemical processes in all eukaryotic organisms (Takeda and Skopick, 1997) and the response of insects to photoperiod was first discovered by Marcovitch in 1923. Longevity of both adult females and larvae was significantly increased when maintained in complete darkness, with survival time being shortest when mites were kept under a short photoperiod. Oviposition rate and the time required for eggs to hatch were also maximal in complete darkness but poorest under a long photoperiod. *Psoroptes* mites do respond to changes in photoperiod but with no fixed pattern, although survival and development is consistently improved under complete darkness. As *Psoroptes* mites spend their entire life on the host (Kirkwood, 1986) at sites, such as the base of the fleece in sheep and the ear of rabbits, they are likely to be in almost complete darkness, it makes sense therefore that light does not play an important role in maintaining events of the mites' life-cycle.

Overall, it is recommended that *Psoroptes* mites are reared in complete darkness at temperatures of between 30 and 32.5°C to maximise the rate of oviposition, the rate of development of the eggs and larval stages, and egg hatch success rate, whilst keeping the survival time of adult female mites at a reasonable level. The results of this study suggest that calf serum can provide enhanced longevity for both adult female mites and larvae, although it does appear to increase the time required for eggs to hatch. Although it is the best of the diets tested here,

calf serum appears not to fulfil the complete nutritional requirements of the mites. Further work is required to determine what other possible diet or environmental conditions are required by larvae to enable them to complete their development to the next stage and increase the possibility of being able to complete the *Psoroptes* life cycle off the host.

CHAPTER 6

DISCUSSION

The aim of the work described in this thesis was to examine aspects of the morphology and behaviour of *Psoroptes* mites with particular reference to their behaviour *in vitro*, to aid with their control and to move closer towards the long-term goal of producing an *in vitro* colony of mites.

The ability to rear *Psoroptes* mites *in vitro* could have a considerable impact on the development of future control methods for the mite. *Psoroptes* mites can currently only be reared successfully on a vertebrate host. This means that any experiments to test new control methods require an artificially infested host. Not only is the maintenance of laboratory animals a welfare issue but it is also very expensive and labour intensive to keep the number of animals required to gain a sufficient sample size. In laboratory-based trials, accurate initial screening could be undertaken using precise numbers of mites in experiments in controlled environmental conditions.

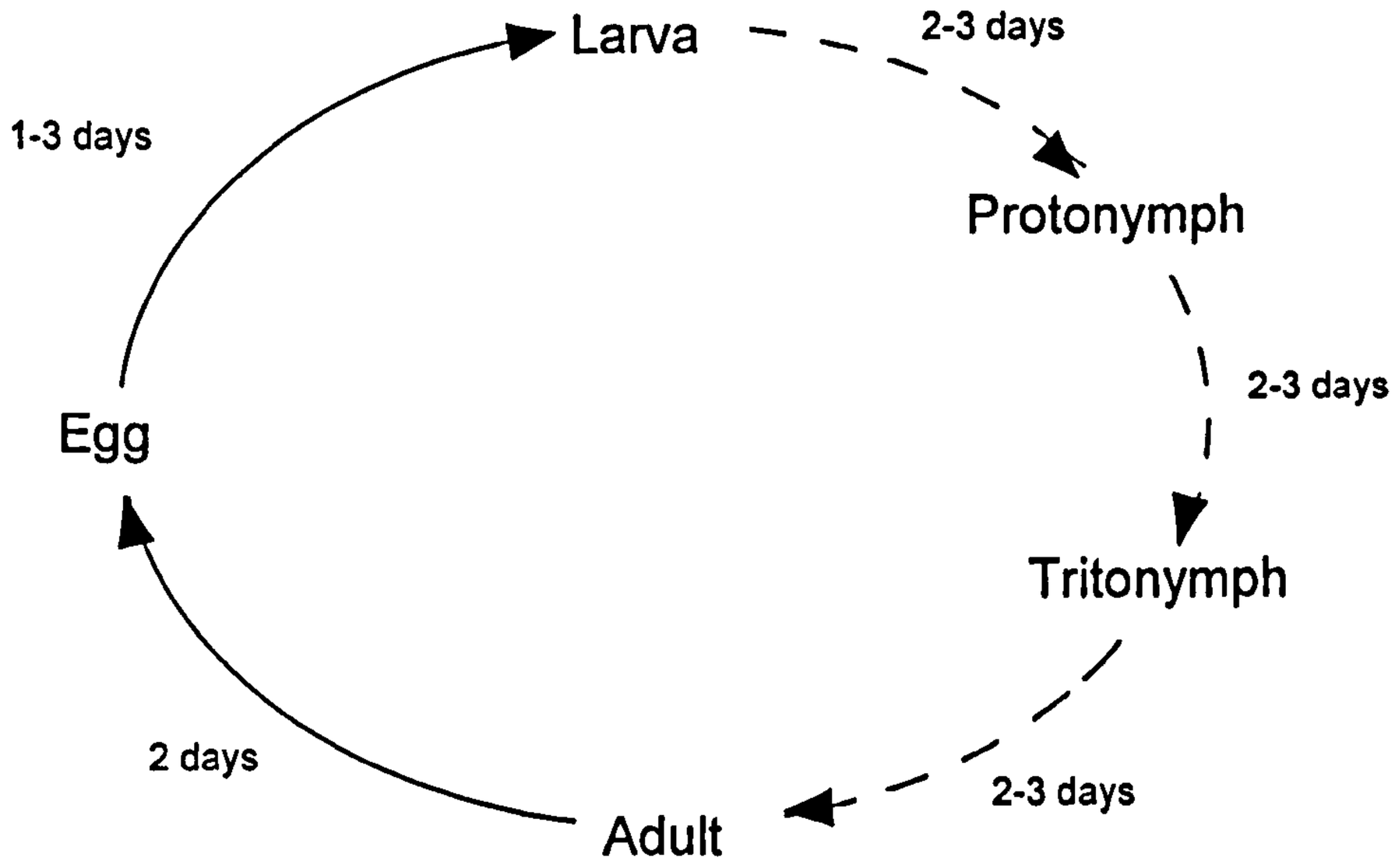
Several studies have examined the off-host survival of *Psoroptes* mites and have concentrated largely on enhancing the longevity of the mites. Mites survive for longer periods of time when maintained at low temperatures with maximum survival of adult female mites recorded as 11.25 days at 10°C (Meintjies *et al.*, 2002c) and 48 days at 5 or 10°C (Liebisch *et al.*, 1985). These studies attempt to maintain mites off-host for as long as the mite is estimated to survive on-host, however, in terms of creating an *in vitro* colony it is not longevity that is the most important element but completion of the life-cycle that is of greatest importance.

The examination of the off-host survival in Chapter 5 shows that the *Psoroptes* lifecycle cannot be completed entirely *in vitro,* although adult females can successfully lay eggs which will hatch and produce larvae (Fig. 6.1). The next step would be to determine what is preventing the moulting of larvae into protonymphs. The lack of a suitable diet may be the cause of failure to moult as moulting has been observed in larvae that have been removed from the host and those may have already fed on-host.

Sterols are required for insects to moult as they are a precursor to moulting hormones (Svoboda, 1999). As many insects are unable to sythesise sterols, they must be present in the diet (Grieneisen, 1994). Cholesterol is the major sterol ingested by carnivorous insects, and may be a requirement for *Psoroptes* mites to allow them to moult successfully. Provision of cholesterol in the media that mites are maintained on may therefore aid their survival and development *in vitro.*

It may also be possible that the presence of adult mites is required for the successful development of the juvenile stages. There may be a component contained within the faeces of adults such as bacteria or enzymes that are needed for the larval stages to fully digest any diet provided to them. It would also be useful to monitor the off-host moult rates of the nymphal stages of the mite. However, as nymphs cannot be reared entirely off-host, they would have to be collected from the host where they may have already fed, and any dietary limitations of moulting would not be noticed.

Figure 6.1 Life-cycle of *Psoroptes* mites. Solid arrows indicate life-cycle stages that have been observed to be completed *in vitro*.



Although *Psoroptes* mites have been observed to feed *in vitro*, as observed in the present work (Chapter 5), DeLoach (1984) failed to increase survival using a glass chamber and a mesh nylon netting feeding surface. Mathieson (1995) examined four different *in vitro* feeding devices and found some success with a Perspex feeding cylinder, where mites fed through nylon gauze placed on a rubber gasket over a reservoir filled with feeding solution; and a plexi-glass feeding device, where a feeding solution soaked piece of filter paper was sandwiched between two pieces of plexi-glass with holes drilled in to create a chamber and covered with a glass coverslip. Using both of these devices, survival was longer than observed in unfed mites kept off-host. However, despite this, it was also noted that egg production was reduced and of the eggs that were laid, none were observed to develop any further than the larval stage.

It is possible that one reason mites are unable to survive *in vitro* is because they cannot properly digest the diet provided or that the diet is nutritionally deficient in some way. It is still not entirely certain what the mites actually feed on. In attempts to rear them off-host, mites have been provided with several host-derived diets including serum, blood, plasma and skin (DeLoach, 1984; Mathieson, 1995) and similar media were tested here (Chapter 5). However, longevity is rarely longer than unfed mites kept off-host, despite demonstration that host-derived products are ingested by *Psoroptes* (Mathieson, 1995). Mathieson (1995) found an apparent increase in longevity in mites that were fed on sheep blood plasma as opposed to whole defibrinated sheep blood and comparing water, plasma, 50% plasma, serum, 50% serum and whole blood, DeLoach (1984) found that significantly fewer mites ingested whole blood compared to the other diets. The greatest feeding response was observed in mites provided with 50% serum, although this was not significant. The effect of a wider range of diets on mite longevity was considered in Chapter 5 but no

consistent improvement in survival times was observed with any particular diet. This suggests that although substances including serum, blood and plasma may be ingested by *Psoroptes*, some other dietary component is absent causing mites maintained *in vitro* on one of these diets to survive little longer than unfed mites.

Using electron microscopy to examine the ultrastructure of the alimentary canal of *Psoroptes*, Mathieson and Lehane (2002) suggested that the mites feed on an almost exclusively liquid diet. Although schlerotized ridges were observed in the pharynx, the presence of bacterial cells and eosinophils in the gut lumen led to the conclusion that these ridges are unlikely to be capable of maceration. It was suggested that the pharyngeal ridges were a vestigial remnant of the mite's ancestors which are likely to have had a different diet.

It has been suggested that *Psoroptes* mites ingest bacteria (Bates, 1991). An examination of the intestinal flora of mites from sheep revealed that *Serratia marcescens* was found in association with the mites (Mathieson and Lehane, 1996) although it was thought to be unlikely that this was acquired as a dietary component as this particular bacterium is not usually found in the natural flora of sheep skin. *Serratia marcescens* has also been isolated from *Psoroptes* mites found on rabbits (Perrucci and Rossi, 2002). In the case of mites from sheep, it was noted that the bacteria were found in sufficiently large numbers such that they may contribute to the nutrition of the mite (Mathieson and Lehane, 1996), something that was found to be the case in the louse *Bovicola ovis* Schrank (Murray and Edwards, 1987). Further work on the bacteria isolated from mites from sheep, using a sequence analysis of genes coding for 16S rRNA, revealed two more bacteria species associated with the mite, one closely related to *Staphylococcus intermedius* or *S. chromogens* and *Alloiococcus otitidis* (Hogg and Lehane, 1999). A further study by Hogg and Lehane (2001) using PCR amplification of 16S rRNA revealed a further seven

species of bacteria associated with *Psoroptes* mites suggesting that there is in fact a very large diversity of bacteria associated with the mite and that there is no consistent species association.

Bates (2003) examined the skin flora of infested and unifested sheep using a 1cm^2 sample of wool taken from the edge of a 48-day-old active sheep scab lesion or from the corresponding position on an unifested sheep. The wool was cultured in nutrient broth and subcultured onto sheep blood agar. The bacteria found on the wool/skin of infested sheep were not the same as the species found on the wool/skin of uninfested sheep providing support for the suggestion of Mathieson and Lehane (1996) that bacteria are carried by the mites and are not derived from the host as a dietary component.

It has been demonstrated that *Psoroptes* mites ingest lipid and that it may make up a major part of the mite's diet (Sinclair and Filan, 1989), however, no lipases were detected in mite extracts (Nisbet and Billingsley, 1999). It has been suggested that mites may ingest bacteria as a means of producing lipases in their guts, enabling them to digest lipids (Hogg and Lehane, 2001). However, Perrucci *et al.* (2001) treated *Psoroptes* mites from rabbits with the antibiotic Amikacin and used them to infest healthy New Zealand rabbits by placing the mites into both of the external auricular meatuses twice, seven days apart and then repeated one month later. All rabbits were found to be infested 70 days after the initial infestation, but only rabbits infested with bacteria-free mites were found to be clinically infested. This suggests that not only are the mites able to survive and infest hosts without the presence of gut bacteria but actually survive better without it. *Serratia marcescens* is the bacterium most commonly isolated from *Psoroptes* mites (Mathieson and Lehane, 1996; Perrucci and Rossi, 2002) and it is known to cause disease in vertebrates including septicaemia (Wijewanta and Fernando, 1970; Mathieson and

Lehane, 1996). As a result of this it has been proposed that it may contribute to the pathology of sheep scab by gaining entry to the host through the lesions (Mathieson and Lehane, 1996). However, *S. marcescens* is also known to be pathogenic towards insect species including the tsetse fly, *Glossina morsitans morsitans* Westwood (Kaaya and Darji, 1989) and the blowfly, *Lucilia sericata* (Meigen)(O'Callaghan *et al.*, 1996) suggesting that the presence of bacteria may actually be detrimental to the survival of the mite.

Proteinases of *P. ovis* have been characterised using substrate gel analysis, inhibitor sensitivity and their ability to degrade protein substrates (Kenyon and Knox, 2002). The proteinases present in *Psoroptes* were able to degrade collagen and fibronectin, blood proteins and immunoglobin G, suggesting roles in lesion initiation, feeding and immuno-evasion, respectively. Proteinases are present that are able to degrade fibrinogen, which is thought to perform an anticoagulant function and therefore prolong serous exudation from the lesion thus aiding mite feeding (Kenyon and Knox, 2002). As fibrinogen was only degraded below pH 5, it may be that the pH at which mites are maintained off-host will have an effect on their longevity. Although it has been suggested that mites may ingest skin cells (Rafferty and Gray, 1987), no proteinases that were able to degrade keratin or elastin were characterised, suggesting that skin is not a major food source (Kenyon and Knox, 2002).

Establishing the precise diet of *Psoroptes* is not only crucial in the development of an *in vitro* rearing system but would also have great implications on the future control of the mite. If the bacteria found associated with the mite are found to be of importance to digestion, application of antibacterial substances may be successful in killing the mite on the host, although initial studies suggest that this would not be successful (Perrucci *et al.*, 2001). Also, whether the mites feed on

host-derived products such as serum or blood will affect the success of systemic control approaches including acaricides and vaccines.

Another potential cause for failure in the rearing of *Psoroptes* mites *in vitro* may be that the mites become damaged during their collection from the host. Thind and Ford (2003) developed a pump-assisted method for collecting mites that utilises a constant flow of pumped saline to remove mites from their host. It was suggested that mites removed in this way had increased fitness, surviving for up to 16 days when incubated at 36°C and 92% r.h. However, it was not stated how many mites were able to survive for this amount of time or whether they were provided with any source of nutrition.

One of the main problems noted by Mathieson (1995) in the development of his *in vitro* feeding system was the climbing and "escape" response that is demonstrated by the mites when they are removed from the host. The data presented in Chapter 4 show that this problem can be overcome because mites respond positively to temperature and therefore a temperature gradient may provide a means to keep mites in contact with the feeding solution in such a system. Maintaining mites on a temperature gradient is also more likely to mimic their natural environment as conditions on the host will not be constant.

It has been demonstrated that *Psoroptes* mites are able to spread rapidly through a group of naïve sheep (Berriatua *et al.*, 1999; Meintjies *et al.*, 2002a) although relatively little is known about the precise methods by which transmission occurs. What actually causes the mites to move to a new host is not known, although it has been observed that the highest rate of transmission occurs at about 9-11 weeks after initial infestation, when the mite population has reached its peak (Berriatua *et al.*, 1999). At this point, it may be that the immune response mounted by the host begins to overcome the mite population. It has been proposed that the scab lesion is

unfavourable to the survival of the mites, causing distortions in body shape (Sinclair and Filan, 1991) and that this may be the reason that mites are found to congregate on the healthy skin around the edge of the lesion. As the lesion spreads over the body of the host, conditions for mite survival may deteriorate forcing the mite to move to a new host.

The data presented in Chapter 4 demonstrate that mites are able to respond to environmental cues including temperature, light and gravity and that these responses are likely to aid transmission and successful initiation of infestation. However, although preliminary studies reported here indicated that at a certain distance apart, there was no apparent interaction between the mites, the presence of pheromones and their potential effects, on mite aggregation for example, was not examined. It is relatively clear how such responses to temperature, light and gravity may aid transmission from the environment when they are off-host, as movement towards the source of heat is likely to bring the mite into contact with a potential host. *Psoroptes* mites are able to survive for relatively long periods of time off-host under field conditions (Stockman, 1912; Wilson *et al.*, 1977; O'Brien *et al.*, 1994a) with maximum recorded survival of 28 days (Wilson *et al.*, 1977). They are also able to initiate a new infection after 12 days off-host, when placed directly on the host (Wilson *et al.*, 1977). However, when already infesting a host, responses to temperature and light, as observed in Chapter 4, would not result in the movement of mites to a new host but might be expected to keep them close to the skin surface.

One of the major threats to any future eradication programmes in the UK may be transmission from other hosts such as domestic rabbits or goats. Successful host transfer experiments, with *Psoroptes* mites from rabbits and goats being used to successfully infest sheep have been reported (Sweatman, 1958) and as has the transfer of mites from rabbits to cattle (Wright, 1982). It has also been shown that

Psoroptes mites from rabbits and sheep are antigenically cross-reactive (Siegfried *et al.*, 2004). The morphological comparison of host-derived populations of *Psoroptes* mites presented in Chapter 3 suggests that *Psoroptes* mites are of a single species but may be phenotypically and possibly physiologically adapted to their local host environment. However, some of the sample sizes of mites obtained from particular host species were quite small and the collection of larger mite samples would greatly strengthen the analysis. Genetic evidence also provides support for the suggestion of a single *Psoroptes* species, with different mite isolates being genotypically highly homologous when examining the sequence data of the second internal transcribed spacer region (ITS-2) of the ribosomal RNA gene (Zahler *et al.*, 1998) and a similar lack of variation when studying the first internal transcribed spacer (ITS-1)(Ramey *et al.*, 2000). This suggests that infestation from other hosts is a possibility. However, *Psoroptes* mites have not yet been found infesting wild rabbit populations in the UK (P. Bates, 1996; R. Wall, unpublished data) although, mites have been observed in the external auditory canals of wild rabbits in France (Guilhon, 1990). This is, perhaps, an area for further investigation, particularly in regions where sheep scab is more prevalent such as Wales, Scotland and Northern England (Bisdorff *et al.*, 2005).

The work described in this thesis has contributed towards the knowledge required to one day establish an *in vitro* colony. It has been demonstrated that mite populations infesting different hosts appear to be of the same species, even if different strains do exist, and this suggests that work carried out on *Psoroptes* mites obtained from different hosts is likely to be comparable. Studies of the behaviour of the mite *in vitro* have suggested that *Psoroptes* may be able to find a new host efficiently using environmental cues and suggests that utilisation of temperature and light gradients may aid the development of an *in vitro* feeding device. Examination

of the longevity of off-host mites has suggested optimal temperatures for survival of adult female mites, oviposition and larval development. However, the main limiting step of completion of the life-cycle off-host appears to be the transition of larvae to the protonymph stage. It may be that diet is the limiting factor, with a major component required for moulting not being provided, or perhaps the presence of adult faeces is required which may be of importance to supply bacteria to aid digestion of the diet provided. Further work is required to address these questions.

REFERENCES

Alekseev, A.N. and Dubinina, H.V. (2000). *Abiotic parameters and diet and seasonal* activity of Borrelia-infected and uninfected *Ixodes persulcatus* (Acarina: Ixodidae. *Journal of Medical Entomology,* 37: 9-15.

Anderson, R.B., Scrimgeour, G.J. and Kaufman W.R. (1998). Responses of the tick *Amblyomma hebraeum* (Acari: Ixodidae), to carbon dioxide. *Experimental and Applied Acarology.* 22: 667-681.

Arlian, L.G., Kaiser, S., Estes, S.A. and Kummel, B. (1981). Infestivity of *Psoroptes cuniculi* in rabbits. *American Journal of Veterinary Research* 42:1782-1784.

Babcock, O. G., and Black, W. L. (1933). The common sheep scab mite and its control. *Texas Agricultural Experiment Station Bulletin* 479: 1-34.

Bates, P.G. (1991). Recent advances in the biology and control of sheep scab. *Proceedings of the Sheep Veterinary Society* 15: 23-27.

Bates, P.G. (1993). Alternative methods for the control of sheep scab. *The Veterinary Record* 133: 467-469.

Bates, P.G. (1996). Epidemiology of subclinical ovine psoroptic otoacariasis in Great Britain. *The Veterinary Record* 138: 388-393.

Bates, P.G. (1997). The pathogenesis and ageing of sheep scab lesions – part 1. *State Veterinary Journal* 7: 11-15.

Bates, P.G. (1999). Inter- and intra-specific variation within the genus *Psoroptes* (Acari: Psoroptidae). *Veterinary Parasitology* 83: 201-217.

Bates, P.G. (2003). Bacterial associations with the sheep scab mite (*Psoroptes ovis*). *Veterinary Record* 152: 206-208.

Bates, P.G. (2004). Therapies for ectoparasiticism in sheep. *In Practice* 26: 538-547.

Bates, P.G., and Groves, B. A. (1991). Failure of a single treatment with ivermectin to control sheep scab (*P. ovis*) on artificially infested sheep. *The Veterinary Record* 128: 250-253.

Bates, P.G., Groves, B. A., Courtney, S. A., and Coles, G. C. (1995). Control of sheep scab (*Psoroptes ovis*) on artificially infested sheep with a single injection of doramectin. *Veterinary Record* 137: 491-492.

Bates, P., and Sayers, R. (2002). The male L_4 outer opisthosomal seta (L_4OOS) of psoroptic mites as a marker for speciation and virulence. Cost Action 833: Mange and Myiasis of livestock Final Conference, University of Bari, Italy. 19-22 September 2002.

Berriatua, E., French, N. P., Wall, R., Smith, K. E., and Morgan, K. L. (1999). Within-flock transmission of sheep scab in naive sheep housed with single infested sheep. *Veterinary Parasitology* 83: 277-289.

Berriatua, E., French, N. P., Broster, C. E., Morgan, K. L., and Wall, R. (2001). Effect of infestation with *Psoroptes ovis* on the nocturnal rubbing and lying behavour of housed sheep. *Applied Animal Behaviour Science* 71: 43-55.

Bisdorff, B., Milnes, A. and Wall, R. (2005). Prevalence and regional distribution of scab, lice and blowfly strike in sheep in Great Britain. *The Veterinary Record* (in press).

Blake, B. H., Bay, D. E., Meola, S. M., and Price, M. A. (1978). Morphology of the mouthparts of the sheep scab mite, *Psoroptes ovis*. *Annals of the Entomological Society of America* 71: 289-294.

Boyce, W., Elliott, L., Clark, R., and Jessup, D. (1990). Morphometric analysis of *Psoroptes spp.* mites from Bighorn sheep, Mule deer, cattle and rabbits. *Journal of Parasitology* 76: 823-828.

Boyce, W. M., and Brown, R. N. (1991). Antigenic characterization of *Psoroptes spp.* (Acari: Psoroptidae) mites from different hosts. *Journal of Parasitology* 77: 675-679.

Brooks, A.J. and Wall, R. (2001). Infection of *Psoroptes* mites with the fungus *Metarhizium anisopliae. Experimental and Applied Acarology* 25: 869-880.

Brooks, A.J., Aquino de Muro, M., Burree, E., Moore, D., Taylor, M.A. and Wall, R. (2004). Growth and pathogenicity of isolates of the fungus *Metarhizium anisopliae* against the parasitic mite, *Psoroptes ovis*: effects of temperature and formulation. *Pest Management Science* 60: 1043-1049.

Broufas, G.D. and Koveos, D.S. (2001). Development, survival and reproduction of *Euseius finlandicus* (Acari: Phytoseiidae) at different constant temperatures. *Experimental and Applied Acarology* 25: 441-460.

Carroll, J.F., Klun, J.A. and Schmidtmann, E.T. (1995). Evidence for kairomonal influence on selection of host-ambushing sites by adult *Ixodes scapularis* (Acari: Ixodidae). *Journal of Medical Entomology.* 32: 119-125.

Chandler, D., Davidson, G., Pell, J.K., Ball, B.V., Shaw, K. and Sunderland, K.D. (2000). Fungal biocontrol of Acari. *Biocontrol Science and Technology* 10: 357-384.

Chavez, C.E. and Guerrero, C.A. (1965). Parasites and parasitic diseases of *Lama pacos* (alpacas) in Peru. Foreign Agricultural Research Grant Project, School of Veterinary Medicine, University of San Marcos, Lima, Peru. Pp1-8.

Clark, A. M., Stephen, F. B., Cawley, G. D., Bellworthy, S. J., and Groves, B. A. (1996). Resistance of the sheep scab mite *Psoroptes ovis* to propetamphos. *Veterinary Record* 139: 451.

Coles, G.C. and Stafford, K.A. (1999). The *in vitro* response of sheep scab mites to pyrethroid insecticides. *Veterinary Parasitology* 83: 327-330.

Corke, M. J. and Broom, D. M. (1999). The behaviour of sheep with sheep scab, *Psoroptes ovis* infestation. *Veterinary Parasitology* 83: 291-300.

Corke, M.J. and Broom, D.M. (2000). Sheep scab: A questionnaire survey of sheep farmers. Proceedings of the Sheep Veterinary Society 24: 115-121.

D'Alterio, G. L., Batty, A., Laxon, K., Duffus, P., and Wall, R. (2001). *Psoroptes* species in alpacas. *The Veterinary Record* 149: 96.

DeLoach, J.R. and Wright, F.C. (1981). Ingestion of rabbit erythrocytes containing ^{51}Cr-labelled haemoglobin by *Psoroptes* spp. (Acari: Psoroptidae) that originated on cattle, mountain sheep or rabbits. *Journal of Medical Entomology* 18: 345-348.

DeLoach, J. R. (1984). In vitro feeding of *Psoroptes ovis* (Acari: Psoroptidae). *Veterinary Parasitology* 16: 117-125.

Dill, R. (1920). Facts about "scab" in sheep. *National Woolgrower* 10: 16.

Downes, B. J. (1990). Host-induced morphology in mites: implications for host-parasite coevolution. *Systematic Zoology* 39: 162-168.

Downing, W. (1936a). The life-history of the scab mite in relation to control measures. *The Veterinary Record* 48: 1065-1067.

Downing, W. (1936b). The life-history of *Psoroptes communis* var. *ovis* with particular reference to latent or suppressed scab. *Journal of Comparative Pathology and Therapeutics* 49: 183-209.

Downing, W. (1947). The control of Psoroptic scab on sheep by benzene hexachloride and DDT. *The Veterinary Record* 59: 581-582.

Fain, A. (1994). Adaptation, specificity and host-parasite coevolution in mites (Acari). *International Journal for Parasitology* 24: 1273-2283.

Fletcher, T. and MacLehose, R. (2005). SHAPE: Survey of Health and Pesticide Exposure. www.vmd.gov.uk

Forbes, A.B., Pitt, S.R., Baggott, D.G., Rehbein, S., Barth, D., Bridi, A.A., Carvalho, L.A. and O'Brien, D.J. (1999). A review of the use of a controlled-release formulation of ivermectin in the treatment and prophylaxis of *Psoroptes ovis* infestations in sheep. *Veterinary Parasitology* 83: 319-326.

Fourie, L.J., Meintjes, T., Kok, D.J. and Horak, I.G. (2002). The growth of sheep scab lesions in relation to sheep breed and time of the year. *Experimental and Applied Acarology* 27: 277-281.

French, N. P., Berriatua, E., Wall, R., Smith, K., and Morgan, K. L. (1999). Sheep scab outbreaks in Great Britain between 1973 and 1992: spatial and temporal patterns. *Veterinary Parasitology* 83: 187-200.

Fürstenburg, M. H. F. (1861). Die Krätmilben der Menschen und Thiere. Verlag von Wilhelm Englemann, Leipzig.

Gervais, M.P. (1841). Sur quelques especes de l'ordre des Acariens. *Annales des Sciences Naturelles Partie Zoologique* 15: 5-10.

Gillespie, A.T. and Moorhouse, E.R. (1989). The use of fungi to control pests of agricultural and horticultural importance. In: Biotechnology of fungi for improving plant growth. Ed. J.M. Whipps and R.D. Lumsden. Cambridge, Cambridge University Press. pp 55-83.

Graham, O.H. and Hourrigan, J.L. (1977). Eradication programs for arthropod parasites of livestock. *Journal of Medical Entomology* 13: 629-658.

Grieneisen, M.L. (1994). Recent advances in our knowledge of ecdysteroid biosynthesis in insects and crustaceans. *Insect Biochemistry and Molecular Biology* 24: 115-132.

Guilhon, J. (1990). Extension corporelle de l'otacariose cuniculine á *Psoroptes cuniculi. Recueil de Médicine Vétérinaire* 166: 119-123.

Guillot, F. S., and Wright, F. C. (1983). Precopulatory pairing and mating of Psoroptes mites (Acari: Psoroptidae). *Journal of Medical Entomology* 20: 591-596.

Hamilton, K.A., Nisbet, A.J., Lehane, M.J., Taylor, M.A. and Billingsley, P.F. (2003). A physiological and biochemical model for digestion in the ectoparasitic mite, *Psoroptes ovis* (Acari: Psoroptidae). *International Journal for Parasitology* 33: 773-785.

Hayes, E.J. and Wall, R. (1999). Age-grading adult insects: a review of techniques. *Physiological Entomology* 24: 1-10.

Hering, E. (1838). Die Kräzmilben der Thiere und einige verwandte Arten, nach eigenen Untersuchungen beschrieben.

Herr, R. (1991). Investigations on resistance of Ribes against the black currant gall mite *Cecidophyopsis ribis* (Westw.)(Acari, Eriophyidae). Bioassays in the laboratory and infestation experiments in the field. *Journal of Applied Entomology* 112: 181-193.

Hirst, S. (1922). Mites injurious to domestic animals. British Museum (Natural History) Economic Series No. 13, London.

Hogg, J.C. and Lehane, M.J. (1999). Identification of bacterial species associated with the sheep scab mite (*Psoroptes ovis*) by using amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology* 65: 4227-4229.

Hogg, J.C. and Lehane, M.J. (2001). Microfloral diversity of cultured and wild strains of *Psoroptes ovis* infesting sheep. *Parasitology* 123:441-446.

Jayawardena, K.G.I., Heller-Haupt, A., Woodland, R.M. and Varma, M.G.R. (1998). Antigens of the sheep scab mite *Psoroptes ovis*. *Folia Parasitologica* 45: 239-244.

Kaaya, G.P. and Darji, N. (1989). Mortality in adult tsetse, *Glossina morsitans morsitans,* caused by entomopathogenic bacteria. *Journal of Invertebrate Pathology* 54: 32-38.

Kenyon, F. and Knox, D. (2002). The proteinases of *Psoroptes ovis*, the sheep scab mite – their diversity and substrate specificity. *Veterinary Parasitology* 105: 317-325.

Kirkwood, A. C. (1980). Effect of *Psoroptes ovis* on the weight of sheep. *The Veterinary Record* 107: 469-470.

Kirkwood, A. C. (1985). Some observations on the biology and control of the sheep scab mite *Psoroptes ovis* (Hering) in Britain. *Veterinary Parasitology* 18: 269-279.

Kirkwood, A. C. (1986). History, biology and control of sheep scab. *Parasitology Today* 2: 302-307.

Kirkwood, A. C., and Bates, P. G. (1987). Flumethrin: A non-stripping pyrethroid dip for the control of sheep scab. *The Veterinary Record* 120: 197-199.

Kirkwood, A. C., and Quick, M. P. (1981). Diazinon for the control of sheep scab. *Veterinary Record* 108: 279-280.

Kirkwood, A. C., and Quick, M. P. (1982). Propetamphos for the control of sheep scab. *The Veterinary Record* 111: 367.

Kopp, K. and Gothe, R. (1995). *Hyalomma truncatum* (Acari: Ixodidae) – investigations into the scototaxis of unfed adult ticks. *Experimental and Applied Acarology.* 19: 53-64.

Lange, R. E., Sandoval, A. V., and Meleney, W. P. (1980). Psoroptic scabies in bighorn sheep (*Ovis canadensis mexicana*) in New Mexico. *Journal of Wildlife Diseases* 16: 77-82.

Lee, A.J., Machell, J., van den Broek, A.H.M., Nisbett, A.J., Miller, H.R.P., Isaac, R.E. and Huntley, J.F. (2002). Identification of an antigen from the sheep scab mite, *Psoroptes ovis*, homologous with house dust mite group I allergens. *Parasite Immunology* 24: 413-422.

Lees, A.D. (1948). The sensory physiology of the sheep tick *Ixodes ricinus. Journal of Experimental Biology* 25: 145.

Li, J., and Margolies, D. C. (1991). Factors affecting location of Banks grass mite, *Oligonychus pratensis* (Acari: Tetranychidae), on corn leaves. *Experimental and Applied Acarology* 12: 27-34.

Liebisch, V. A., Olbrich, S., and Deppe, M. (1985). Untersuchungen zur Uerlebensdauer von Milben der Arten *Psoroptes cuniculi* und *Chorioptes bovis* abseits des belbten Wirtes. *Dtsch. tierarztl. Wschr.* 92: 181-185.

Loxam, J. G. (1974). Sheep scab epidemic: January 1973. *State Veterinary Journal* 29: 1-10.

Macchioni, F., Perrucci, S., Cecchi, F. Cioni, P.L., Morelli, I. and Pampiglione, S. (2004). Acaricidal activity of aqueous extracts of camomile flowers, *Matricaria chamomilla,* against the mite *Psoroptes cuniculi. Medical and Veterinary Entomology* 18: 205-207.

MacInnis, A.J. (1976). How parasites find hosts: some thoughts on the inception of hosts-parasite integration. In: Kennedy, C.R. (ed.) Ecological Aspects of Parasitology. North-Holland Publishing Co., Amsterdam, The Netherlands, pp. 3-20.

Mapstone, S.C., Beasley, A. and Wall, R. (2002). Structure and function of the gnathosoma of the mange mite, *Psoroptes ovis*. *Medical and Veterinary Entomology* 16: 378-385.

Marcovitch, S. (1923). Plant lice and light exposure. *Science* 58: 537-538.

Mathieson, B.R.F. (1995). An investigation of *Psoroptes ovis*, the sheep scab mite, with a view to developing an *in vitro* feeding system. PhD Thesis, University of Wales, Bangor.

Mathieson, B. R. F. and Lehane, M.J. (1996). Isolation of the Gram-negative bacterium, *Serratia marcescens*, from the sheep scab mite, *Psoroptes ovis*. *The Veterinary Record* 138: 210-211.

Mathieson, B.R.F. and Lehane, M.J. (2002). Ultrastructure of the alimentary canal of the sheep scab mite, *Psoroptes ovis* (Acari: Psoroptidae). *Veterinary Parasitology* 104: 151-166.

McMahon, C. and Guerin, P.M. (2002). Attraction of the tropical bont tick, *Amblyomma variegatum*, to human breath and to the breath components acetone, NO and CO_2. *Naturwissenschaften* 89: 311-315.

McPherson, M., Shostak, A.W. and Samuel, W.M. (2000). Climbing simulated vegetaion to heights of ungulate hosts by larvae of *Dermacentor albipictus* (Acari: Ixodidae). *Journal of Medical Entomology*. 37: 114-120.

Megnin, J.P. (1877). Monographie de la tribu des Sarcoptides Psoriques qui comprend tous les acariens de la gale de l'homme et des animaux. *Revue et Magasin de Zoologie*, 46-139.

Meintjes, T., Fourie, L.J. and Horak, I.G. (2002a). The rate of spread of sheep scab within small groups of Merino and Dorper sheep. *Journal of the South African Veterinary Association* 73: 137-138.

Meintjes, T., Fourie, L.J. and Horak, I.G. (2002b). Host preference of the sheep scab mite, *Psoroptes ovis*. *Journal of the South African Veterinary Association* 73: 135-136.

Meintjes, T., Fourie, L.J. and Horak, I.G. (2002c). On-host ecology and off-host survival of the sheep scab mite *Psoroptes ovis*. *Onderstepoort Journal of Veterinary Research* 69: 273-283.

Meleney, W. P. (1967). Experimentally Induced Bovine Psoroptic Acariasis in a Rabbit. *American Journal of Veterinary Research* 28: 892-894.

Meleney, W.P. and Christy, J.E. (1978). Factors complicating control of psoroptic scabies of cattle. *Journal of the American Veterinary Medical Association* 173: 1473-1478.

Murray, M.D. and Edwards, J.E. (1987). Bacteria in the food of the biting louse of sheep, *Damalinia ovis*. *Australian Veterinary Journal* 64: 277-278.

Nisbet, A.J. and Billingsley, P.F. (1999). Hydrolytic enzymes of *Psoroptes cuniculi* (Delafond). *Insect Biochemistry and Molecular Biology* 29: 25-32.

Nordenfors, H., Höglund, J. and Uggla, A. (1999). Effects of temperature and humidity on oviposition, molting and longevity of *Dermanyssus gallinae* (Acari: Dermanyssidae). *Journal of Medical Entomology* 36: 68-72.

O'Brien, D. J., Gray, J. S., and O'Reilly, P. F. (1994a). Survival and retention of infectivity of the mite *Psoroptes ovis* off the host. *Veterinary Research Communications* 18: 27-36.

O'Brien, D. J., Gray, J. S., and O'Reilly, P. F. (1994b). The use of moxidectin 1% injectable for the control of psoroptic mange in sheep. *Veterinary Parasitology* 52: 91-96.

O'Brien, D. J. (1999). Treatment of psoroptic mange with reference to epidemiology and history. *Veterinary Parasitology* 83: 177-185.

O'Callaghan, M., Garnham, M.L., Nelson, T.L., Baird, D. and Jackson, T.A. (1996). The pathogenicity of *Serratia* strains to *Lucilia sericata. Journal of Invertebrate Pathology* 68: 22-27.

Ochs, H., Mathis, A., and Deplazes, P. (1999). Single nucleotide variation in rDNA ITS-2 differentiates *Psoroptes* isolates from sheep and rabbits from the same geographical area. *Parasitology* 119: 419-424.

O'Nuallain, T.O. (1966). Psoroptic acariasis or sheep scab; a review. *Irish Veterinary Journal* 20: 187-193.

Otranto, D., Milillo, P., Mesto, P., De Caprariis, D., Perrucci, S., and Capelli, G. (2004). *Otodectes cynotis* (Acari: Psoroptidae): examination of survival off-the-host under natural and laboratory conditions. *Experimental and Applied Acarology* 32: 171-179.

Owen, J.P., and Mullens, B.A. (2004). Influence of heat and vibration on the movement of the northern fowl mite (Acari: Macronyssidae). *Journal of Medical Entomology.* 41: 864-872.

Perrucci, S., Cioni, P.L., Flamini, G., Morelli, I. and Macchioni, G. (1995). Structure/activity relationship of some natural monoterpenes as acaricides against *Psoroptes cuniculi. Journal of Natural Products.* 58: 1261-1264.

Perrucci, S., Macchioni, G., Cioni, P.L., Flamini, G., Taccini, F. and Morelli, I. (1996). The activity of volatile compounds from *Lavendula angustifolia* against *Psoroptes cuniculi. Phytotherapy Research* 10: 5-8.

Perrucci, S., Rossi, G., Macchioni, G. and O'Brien, D.J. (2001). The influence of internal bacterial flora on the virulence of *Psoroptes cuniculi*. Cost Action 833 Agriculture (Mange and Myiasis in livestock). 4th Annual Meeting, National Veterinary School, Toulouse, France, October 3 to 6, 2001.

Perrucci, S. and Rossi, G. (2002). Aerobic and microaerophilic bacteria isolated from *Psoroptes cuniculi*. *Parassitologia* 44: 149-151.

Pilkington, A., Buchanan, D., Jamal, G.A., Gillham, R., Hansen, S., Kidd, M., Hurley, J.F. and Soutar, C.A. (2001). An epidemiological study of the relations between exposure to organophosphate pesticides and indices of chronic peripheral neuropathy and neuropsychological abnormalities in sheep farmers and dippers. *Occupational and Environmental Medicine* 58: 702-710.

Pruett, J.H. (1999). Identification and purification of a 16-kDa allergen from *Psoroptes ovis* (Acarina: Psoroptidae) *Journal of Medical Entomology* 36: 544-550.

Rafferty, D. E., and Gray, J. S. (1987). The feeding behaviour of *Psoroptes spp.* mites on rabbits and sheep. *Journal of Parasitology* 73: 901-906.

Raillet, A. (1893). Traite de zoologie medicale et agricole. Part 1. Asselin et Houzeau, Paris.

Ramey, R.R., Kelley, S.T., Boyce, W.M. and Farrell, B.D. (2000). Phlogeny and host specificity of Psoroptic mange mites (Acarina: Psoroptidae) as indicated by ITS sequence data. *Journal of Medical Entomology.* 37: 791-796.

Rehbein, S., Visser, M., Winterm R. and Maciel, A.E. (2002). Efficacy of a new long-acting formulation of ivermectin and other injectable avermectins against induced *Psoroptes ovis* infestations in cattle. *Parasitology Research* 88: 1061-1065.

Reese, N. E., Boyce, W. M., Gardner, I. A., and Nelson, D. M. (1996). Fixation affects morphometric characters of *Psoroptes cuniculi* mites (Acari: Psoroptidae). *Journal of Medical Entomology* 33: 835-838.

Roberts, I. H., and Meleney, W. P. (1971). Variations among strains of *Psoroptes ovis* (Acarina: Psoroptidae) on sheep and cattle. *Annals of the Entomological Society of America* 64: 109-116.

Salmon, D.E. and Stiles, C.H. (1903). Scab in sheep. *United States Department of Agriculture Farmers Bulletin* 159: 7-47.

Sanders, A., Froggatt, P., Wall, R., and Smith, K. E. (2000). Life-cycle stage morphology of *Psoroptes* mange mites. *Medical and Veterinary Entomology* 14: 131-141.

Sargison, N. D., Scott, P. R., Penny, C. D., and Pirie, R. S. (1995). Effect of an outbreak of sheep scab (*Psoroptes ovis* infestation) during mid-pregnancy on ewe body condition and lamb birthweight. *The Veterinary Record* 136: 287-289.

Seddon, H. R. (1964). Eradication of sheep scab from New South Wales. *Australian Veterinary Journal* 40: 418-421.

Shilston, A. W. (1915). Sheep scab. Observatons on the life-history of *Psoroptes communis* var *ovis* , and some points connected with the epizootiology of the disease in South Africa. In: The Third and Fourth Reports of the Director of Veterinary Research, pp. 70-98. Department of Agriculture, Union of South Africa, Pretoria.

Siegfried, E., Ochs, H., and Deplazes, P. (2004). Clinical development and serological antibody responses in sheep and rabbits experimentally infested with *Psoroptes ovis* and *Psoroptes cuniculi*. *Veterinary Parasitology* 124: 109-124.

Sinclair, A.N. and Filan, S.J. (1989). Lipid ingestion from sheep epidermis by *Psoroptes ovis* (Acari: Psoroptidae). *Veterinary Parasitology* 31: 149-164.

Sinclair, A. N. and Filan, S. J. (1991). Confirmation of degenerative effects on psoroptic mites from scab lesions. *The Veterinary Record* 129: 492.

Sinclair, A. N. and Kirkwood, A. C. (1983). Feeding behaviour of *Psoroptes ovis*. *The Veterinary Record* 112: 65.

Smith, K. E., Wall, R., Berriatua, E., and French, N. P. (1999). The effects of temperature and humidity on the off-host survival of *Psoroptes ovis* and *Psoroptes cuniculi*. *Veterinary Parasitology* 83: 265-275.

Smith, K.E., Wall, R. and French, N.P. (2000). The use of entomopathogenic fungi for the control of parasitic mites, *Psoroptes* spp. *Veterinary Parasitology* 92: 97-105.

Smith, W. D., Bates, P., Pettit, D. M., Van den Broek, A., and Taylor, M. A. (2002). Attempts to immunize sheep against the scab mite, *Psoroptes ovis*. *Parasite Immunology* 24: 303-310.

Smith, W. D., and Pettit, D. M. (2004). Immunization against sheep scab: preliminary identification of fractions of *Psoroptes ovis* which confer protective effects. *Parasite Immunology* 26: 307-314.

Soll, M. D., Carmichael, I. H., Swan, S. E., and Abrey, A. (1992). Treatment and control of sheep scab (*Psoroptes ovis*) with ivermectin under field conditioning in South Africa. *The Veterinary Record* 130: 572-574.

Stephens, R., Spurgeon, A., Calvert, I.A., Beach, J., Levy, L.S., Berry, H. and Harrington, J.M. (1995). Neurophsychological effects of long-term exposure to organophosphates in sheep dip. *Lancet* 345: 1135-1139.

Stockman, S. (1910). Some points on the epizootiology of sheep scab in relation to eradication. *Journal of Comparative Pathology and Therapeutics* 23: 303-304.

Stockman, S. (1912). Experimental work on sheep scab. *The Veterinary News* Aug. 24, pp. 405-409.

Stockman, S., and Berry, A. H. (1913). The *Psoroptes communis ovis*: Some observations on ova and ovipositing. *Journal of Comparative Pathology and Therapeutics* 26: 45-50.

Strong, K. L., and Halliday, R. B. (1992). Biology and host specificity of the genus *Psoroptes* Gervais (Acarina: Psoroptidae), with reference to its occurrence in Australia. *Experimental and Applied Acarology* 15: 153-169.

Svoboda, J.A. (1999). Variability of metabolism and function in sterols in insects. *Critical Reviews in Biochemistry and Molecular Biology* 34:49-57.

Sweatman, G. K. (1958). On the life history and validity of the species in Psoroptes, a genus of mange mites. *Canadian Journal of Zoology* 36: 905-929.

Synge, B. A., Bates, P. G., Clark, A. M., and Stephen, F. B. (1995). Apparent resistance of *P. ovis* to flumethrin. *The Veterinary Record* 137: 51.

Takeda, M. and Skopik, S.D. (1997). Photoperiodic time measurement and related physiological mechanisms in insects and mites. *Annual Review of Entomology* 42: 323-349.

Tarry, D. W. (1974). Sheep scab: Its diagnosis and biology. *The Veterinary Record* 95: 530-532.

Thind, B.B. and Ford, H.L. (2003). A simple pump-assisted method for collecting live, undamaged *Psoroptes ovis* from sheep using circulating saline. *Veterinary Parasitology* 114: 215-222.

Tovey, E. R., Chapman, M. D., and Platts-Mills, T. A. E. (1981). Mite faeces are a major source of house dust allergens. *Nature* 289: 592-593.

Vail, S.G., and Smith, G. (1998). Air temperature and relative humidity effects on behavioural activity of blacklegged tick (Acari: Ixodidae) nymphs in New Jersey. *Journal of Medical Entomology.* 36: 1025-1028.

Van den Broek, A.H. and Huntley, J.F. (2003). Sheep Scab: the disease, pathogenesis and control. *Journal of Comparative Pathology* 128: 79-91.

Van den Broek, A. H. M., Huntley, J. F., Machell, J., Taylor, M., Bates, P., Groves, B. and Miller, H. R. P. (2000). Cutaneous and systemic responses during primary and challange infestations of sheep with the sheep scab mite, *Psoroptes ovis*. *Parasite Immunology* 22: 407-414.

Wall, R., French, N., and Morgan, K. (1992). Effects of temperature on the development and abundance of the sheep blowfly *Lucilia sericata*. *Bulletin of Entomological Research* 82: 125-131.

Wall, R., Smith, K. E., Berriatua, E., and French, N. P. (1999). Simulation analysis of the population dynamics of the mite, *Psoroptes ovis*, infesting sheep. *Veterinary Parasitology* 83: 253-264.

Wijewanta, E.A. and Fernanado, M. (1970). Infection in goats owing to *Serratia marcescens*. *The Veterinary Record* 87: 282-284.

Wilson, G. I., Blachut, K., and Roberts, I. H. (1977). The infectivity of scabies (mange) mites, *Psoroptes ovis* (Acarina: Psoroptidae), to sheep in naturally contaminated enclosures. *Research in Veterinary Science* 22: 292-297.

Wright, F. C. (1982). Rearing of *Psoroptes cuniculi* Delafond on Cattle. *The Southwestern Entomologist* 7: 235-239.

Wright, F. C., Riner, J. C., and Guillot, F. S. (1983). Cross-mating studies with *Psoroptes ovis* (Hering) and *Psoroptes cuniculi* Delafond (Acarina: Psoroptidae). *Journal of Parasitology* 69: 696-700.

Wright, F. C., Riner, J. C., and Fisher, W. F. (1984). Comparison of lengths of outer opisthosomal setae of male Psoroptic mites collected from various hosts. *Journal of Parasitology* 70: 141-143.

Zahler, M. and Gothe, R. (1995). Effect of temperature and humidity on longevity of unfed adults and on oviposition of engorged females of *Dermacentor reticulatus* (Ixodidae). *Applied Parasitology* 36: 200-211.

Zahler, M., Essig, A., Gothe, R., and Rinder, H. (1998). Genetic evidence suggests that *Psoroptes* isolates of different phenotypes, hosts and geographic origins are conspecific. *International Journal for Parasitology* 28: 1713-1719.

Zahler, M., Hendrikx, W. M. L., Essig, A., Rinder, H., and Gothe, R. (2000). Species of the genus *Psoroptes* (Acari: Psoroptidae): A taxonomic consideration. *Experimental and Applied Acarology* 24: 213-225.

Experimental and Applied Acarology **33:** 69–79, 2004.

Tactic responses of the parasitic mite, *Psoroptes ovis*, to light and temperature

K.R. PEGLER* and R. WALL
*School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG, UK; *Author for correspondence (e-mail: k.r.pegler@bristol.ac.uk; phone: +44-117-928-7489)*

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Abstract. The astigmatid mite, *Psoroptes ovis* (Hering) (Acari: Psoroptidae), is an obligate, non-burrowing ectoparasite of vertebrates, of particular economic importance in domestic sheep flocks where it causes clinical psoroptic mange. To help understand the behaviour which facilitates transmission via the environment, the responses of *P. ovis* derived from rabbits (syn. *Psoroptes cuniculi*) to temperature and light were examined in the laboratory. On a vertical surface of uniform temperature, the presence and direction of illumination had a significant effect on the distance and direction moved by the mites. In darkness or with illumination from both above and below, the mites moved relatively little, but this movement was upwards. In contrast, with illumination from above only, mites moved downwards. When the direction of the illumination was reversed so that it came from below only, the mites moved upwards. On a vertical surface with a temperature gradient, in darkness or with illumination from both above and below, the mites moved up or down towards the area of highest temperature, depending on whether this was above or below, respectively. However, the movement of the mites in response to the temperature gradient was strongly displaced up or down by the presence of unidirectional illumination from above or below, respectively. The results indicate that the movement of these mites is strongly directed towards areas of high temperature but away from higher light intensity. These behaviours might be expected to maintain the position of the mites on a host animal and help them locate the skin surface of a new host when displaced into the environment.

Introduction

Many species of tick and mite display pronounced tactic and kinetic behaviours in response to chemical, temperature and gravitational cues, which can allow them to make rapid contact with their host or food source. For parasitic species in particular, the period over which many species can survive off their host or can undertake active searching is often restricted to periods of only a few days and host-location behaviour must therefore be precise. For example, specific chemical cues, including animal urine, breath and gland secretions are used by several tick species to locate their host (Carroll et al. 1995). Tarsal gland secretions and urine of white-tailed deer were found to produce an arrestant response in female *Ixodes scapularis* Say (Acari: Ixodidae) and the ticks became stationary on portions of glass tubing coated with the substances (Carroll et al. 1995). Similarly, the ixodid ticks *Amblyomma variegatum* (Fabricius), *Rhipicephalus sanguineus* Latreille, *Ixodes ricinus* Linnaeus and the argasid *Ornithodorous moubata* (Murray) all show attraction towards diluted human breath when placed in an air stream (McMahon and Guerin 2002). Attraction towards three of the individual components of human breath; acetone,

nitrogen oxide and carbon dioxide has also been demonstrated, although a reduced speed of locomotion towards the source of the chemical was observed in the case of acetone and nitrogen oxide. The response shown to a particular substance may vary depending upon the life-cycle stage; *Amblyomma hebraeum* (Koch) shows enhanced responsiveness to carbon dioxide during the host-seeking periods of the life-cycle, with the amount of questing increasing over the 6 week period following moulting (Anderson et al. 1998). In addition to kairomones, temperature, light intensity and visual cues are also known to be used by mites and ticks.

The astigmatid mite, *Psoroptes ovis* (Hering) (Acari: Psoroptidae), is an obligate, non-burrowing ectoparasite of vertebrates. It infests a range of hosts, including sheep, goats and cattle. Its presence may trigger a hypersensitivity response, possibly in response to antigens found in the faeces, leading to an acute clinical disease, known as psoroptic mange (Sinclair and Kirkwood 1983; Mathieson and Lehane 1996). Symptoms of infestation may include surface exudation, skin inflammation and severe irritation causing the host to become restless and to scratch and bite at the infested area and may result in the death of the host within 8–12 weeks (Berriatua et al. 1999, 2001). Psoroptic mange is common throughout Europe and other parts of the world, such as South America (French et al. 1999; Corke and Broom 2000; Falconi et al. 2002).

Transmission of *P. ovis* may occur directly between infested and uninfested hosts (Wilson et al. 1977; Berriatua et al. 1999, 2001). The probability of transmission from infested to naive hosts has been shown to increase over the period following initial infestation in line with changes in mite abundance, but not to be related significantly to the rubbing activity of infested animals (Berriatua et al. 1999). Transmission may also occur via the environment, with most estimates suggesting an off-host survival time and a period over which infestation remains possible from a contaminated environment, of 15–20 days (O'Brien et al. 1994; Smith et al. 1999; Meintjies et al. 2002). Whether changes in the behaviour of the mites, such as dispersal activity, might contribute to increasing the probability of transmission, either from infested hosts or via the environment, is currently unknown. Detailed studies of this aspect of mite behaviour, however, may allow a more comprehensive understanding of the process of infection and, furthermore, may contribute towards the successful maintenance of mites *in vitro*. The ability to rear *P. ovis* off-host has been a long-standing research objective (Mathieson 1995), since mites can currently only be reared on a live vertebrate host and an *in vitro* colony would therefore be of considerable practical research value. The aim of the work described here, therefore, was to examine the responses *P. ovis* mites *in vitro* to temperature gradients and illumination.

Materials and methods

Mites

Psoroptes ovis mites were collected from the ears of infested rabbits in scabs. Conventionally, *Psoroptes* infesting the ears of rabbits have been described as

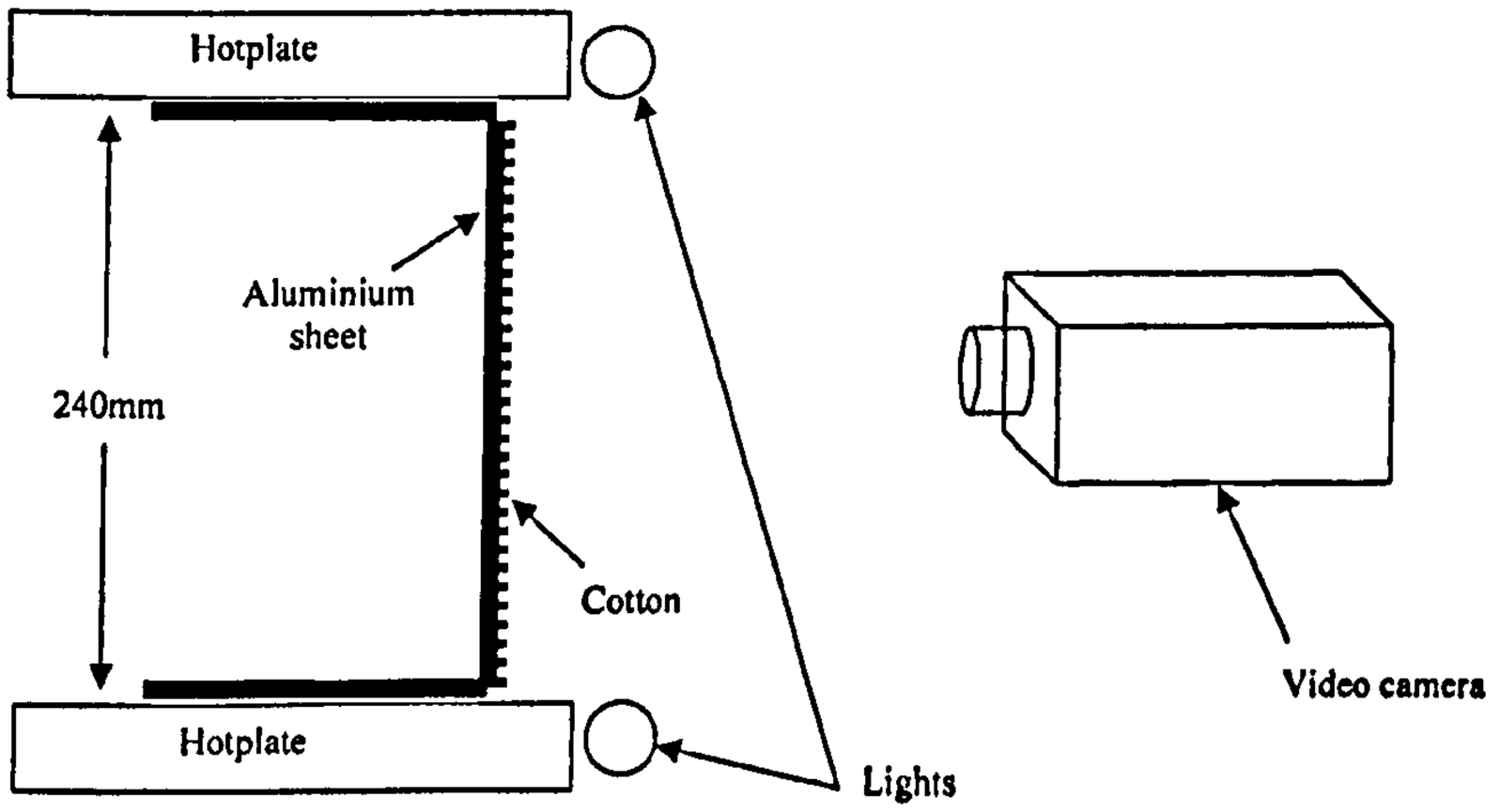

Figure 1. Aluminium sheet arena, with hot-plates and fluorescent lights, used to create temperature and light gradients. A video camera and recorder were used to record movement of the mite *P. ovis*.

Psoroptes cuniculi (Sweatman 1958). However, recent studies do not support a phylogenetic distinction between the *Psoroptes* isolated from sheep and rabbits (Zahler et al. 1998; Bates 1999; Sanders et al. 2000; Evans et al. 2003). The name *P. ovis* has taxonomic priority and, hence, the mites used in the present study will be described as *P. ovis* derived from rabbits.

Adult female mites were removed from the scab as required. Only adult female mites were used because this is the most long-lived life-cycle stage and, as the reproductive stage, is the most critical in terms of population dynamics. Each mite was used only once and mites were not used when they had been off the host for more than 48 h. Between trials, mites were kept in a refrigerator at approximately 4 °C. Before each trial, scabs and mites were removed from the refrigerator and maintained at 25 °C for approximately 30–60 min, allowing them to adjust room temperature gradually, before use.

Apparatus

To monitor the behaviour of the mites, an arena was constructed to provide a surface for the mites to walk over (Figure 1). For this, a sheet of aluminium (450 mm × 165 mm × 1.2 mm) was bent so that it presented a flat vertical surface (240 mm × 165 mm) with two (105 mm × 165 mm) projections at its top and bottom, bent at 90° to the front surface. Two thermostatically controlled hotplates (Dishwarmer 2, 220–250 V 170 W, Photax, UK) were attached to the top and bottom projections of the aluminium sheet. The hotplates allowed a temperature gradient to be produced across the longitudinal axis of the arena. Two 15 W fluorescent lights, 420 mm in length and 25 mm diameter (Duro-lite®, True-lite,

Illinois, USA) were also clamped at the top and bottom of the arena surface to allow a light gradient to be produced.

A rectangular (240 mm × 165 mm) piece of white cotton (Arcade Sewing Machine Co. Ltd., Bristol, UK) was attached to the front surface of the arena. A line was drawn horizontally across its centre. Prior to each trial, the sheet of cotton was immersed in water and then excess water wrung out to provide a dampened surface. To standardise and, if necessary, correct for differences in wetness, the cotton was weighed dry and wet the at the start of each trial and the difference calculated.

All the apparatus, arena, hotplates and lights, were then placed inside a larger aluminium outer chamber. The lid of the chamber could be closed to exclude any exterior light. Air was sucked into the chamber and through a filter of activated charcoal by a 10 cm diameter fan, to eliminate any organic sources of odour that might have affected the behaviour of the mites. A small perspex window in the lid of the chamber allowed a video camera (Swallow, MCD 2075, Custom Cameras Ltd, Wells, UK) to record the movements of the mites from a distance of 60 mm.

Three adult female *P. ovis* mites were used in each trial. The mites were placed approximately 5 cm apart and, at this distance, there was no apparent interaction between individuals. They were removed from the scab and placed on the centre line of the piece of cotton. After closing the lid on the aluminium outer chamber, the video camera was turned on and the movements of the mites were recorded for 5 min.

Temperature and light gradients

Temperature and light gradients were created across the test arena using the hotplates and fluorescent lights. To produce a temperature gradient, one of the hotplates was turned on at a temperature between 30 and 50 °C while the other one was turned off. The gradient was allowed to establish for at least 20 min before the experiment commenced. Temperature was measured at five evenly distributed points along the vertical axis of the test arena and was recorded at the start, middle and end of each 5 min trial. Room temperature was maintained using a thermostatically controlled fan heater; average room temperature over the course of the trials described here was recorded as 25.9 ± 0.1 °C. As a control, neither of the hotplates were turned on so that no temperature gradient was present.

Trials were run with either both fluorescent lights turned on or, to produce a light gradient, with only one of the fluorescent lights, either top or bottom, turned on. It was assumed that with only one light turned on, light intensity declined detectably from the light source over the surface of the test arena, although this was not measured. Controls were run with both lights turned off, in which case the video camera was not used; trials were carried out in total darkness and at the end of 5 min the lights were switched on and the position of the mites was marked on the cotton surface.

Each of the 28 light and temperature combinations were run six times, each with three mites. These light and temperature combinations consisted of seven different

temperature gradients (both hotplates turned off, or either top or bottom hotplate set at 31.1 ± 0.1, 39.5 ± 0.2, 48.35 ± 0.1 °C) and four different light combinations (both lights turned on, both lights turned off or either top or bottom light turned on only). These combinations meant that the effects of temperature and light could be looked at individually or together. Ambient room relative humidity was recorded at the start of each trial and varied little during the experimental work reported here (mean $= 47.2 \pm 0.5\%$ r.h.).

Analysis

The video recordings of the mite movements were displayed on a computer screen and their positions digitised as X–Y co-ordinates and recorded every 20 s. Vertical distance travelled, which is the primary variable considered here, is defined as the straight line distance travelled perpendicular to the line of origin. A positive value therefore indicates an upward movement and a negative value indicates a downward movement. The measures of vertical distance travelled were tested for normality and treated either as the dependent variable in analysis of variance where light treatment (on/off) was a factor, or as the dependent variable in linear or polynomial regression, where temperature difference was the independent variable. Tukey multiple range tests were used to explore the differences between classification groups following analysis of variance.

Results

Multiple regression analysis was used to first consider the possibility that environmental factors, which were not controlled in the trials, might have had a significant impact on the distances moved by the mites. For all trials, the distance moved was regressed against the ambient humidity and temperature of the laboratory, the water content of the cotton covering the test arena and the absolute temperature of the hotplate that was not turned on. No consistent significant effect of any of these factors was found and it was concluded therefore that they had no impact on the results and were not considered further.

Temperature and light gradients

The presence and direction of the illumination had a significant effect on the distance and direction moved by the mites ($F_{62} = 8.41$, $P = 0.001$) (Figure 2). With no temperature gradient and both lights on or both off, so that there was no unidirectional light gradient, the mites moved relatively little and there was no significant difference in the distance moved between these two light treatments (Tukey multiple range test, $P > 0.05$). There was, however, a slight tendency to move upwards ($t = 2.28$, $P = 0.03$). In contrast, when the illumination was from above

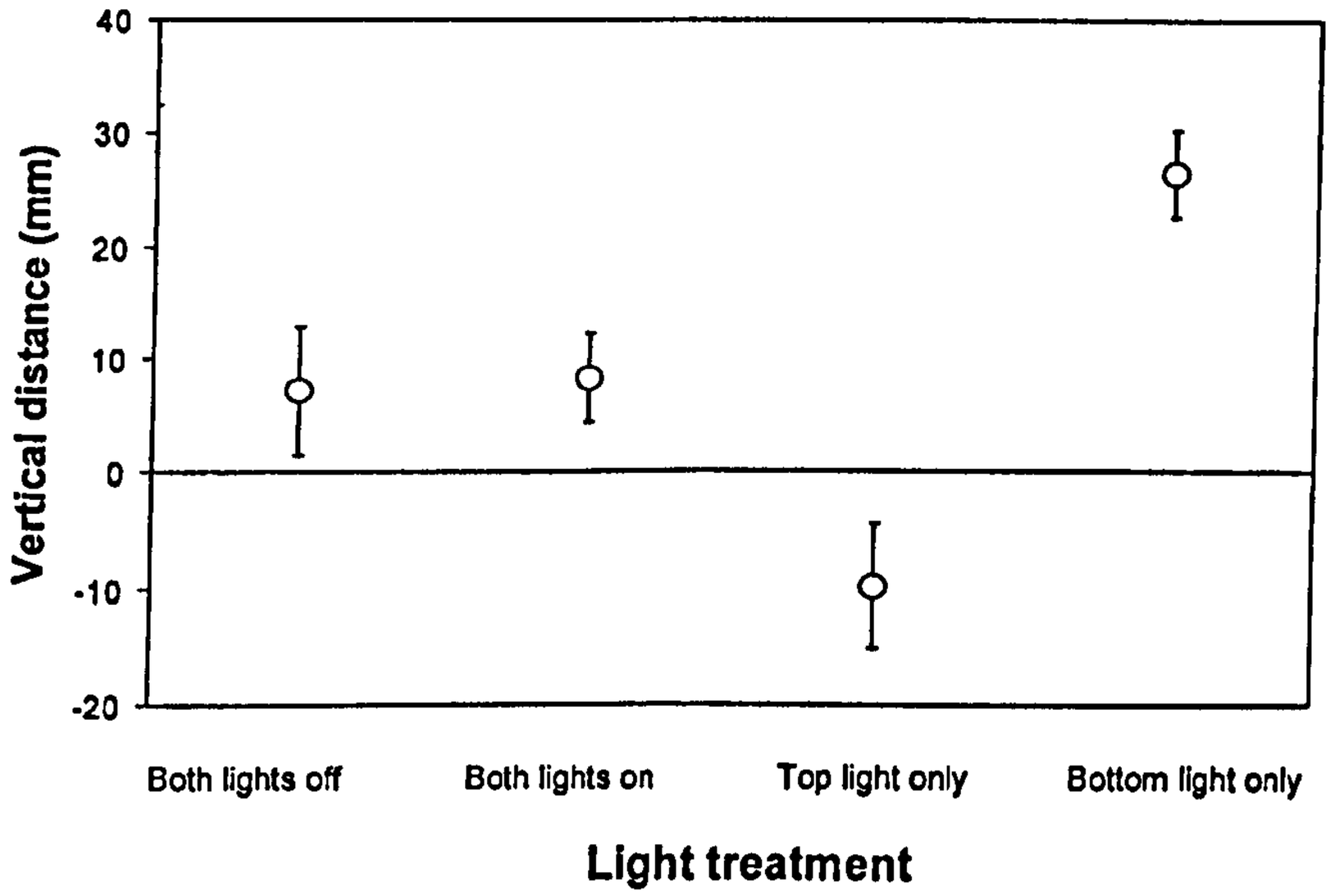

Figure 2. The mean distance moved by *P. ovis* mites when subjected to unidirectional or non-unidirectional light gradients.

only the mites moved down below the line of origin, but when illumination was from below only the mites moved upwards. The distances moved when the arena was illuminated from above or below only were significantly different (Tukey multiple range test, $P<0.05$) and both were significantly different from the distances moved with either both or no lights turned on (Tukey multiple range test, $P<0.05$). The data show that the mites moved away from the source of the illumination when this was unidirectional.

With a temperature gradient and both lights turned on, there was a significant relationship between the vertical distance moved and temperature difference between the top and bottom of the arena (Figure 3) ($F_{115}=55.4$, $P<0.001$, $r^2=32.7\%$). As the gradient became greater and hotter at the top, the mites moved upwards; when the gradient became greater and hotter at the bottom, the mites moved down. Hence, the mites consistently moved towards the area of highest temperature, although, as shown, the variance around the fitted regression line is relatively high. When this trial was repeated with no lights on, a similar significant but highly variable relationship was found ($F_{124}=14.7$, $P<0.001$, $r^2=10.7\%$). Neither the slopes nor intercepts of the two regressions differed significantly, suggesting that the mites responded in the same way to the temperature gradient, regardless of whether the lights were on or off.

When illumination was from above only, the movement of the mites in response to the temperature gradient was displaced downwards with respect to the origin (Figure 4a). However, there was still a significant positive polynomial relationship

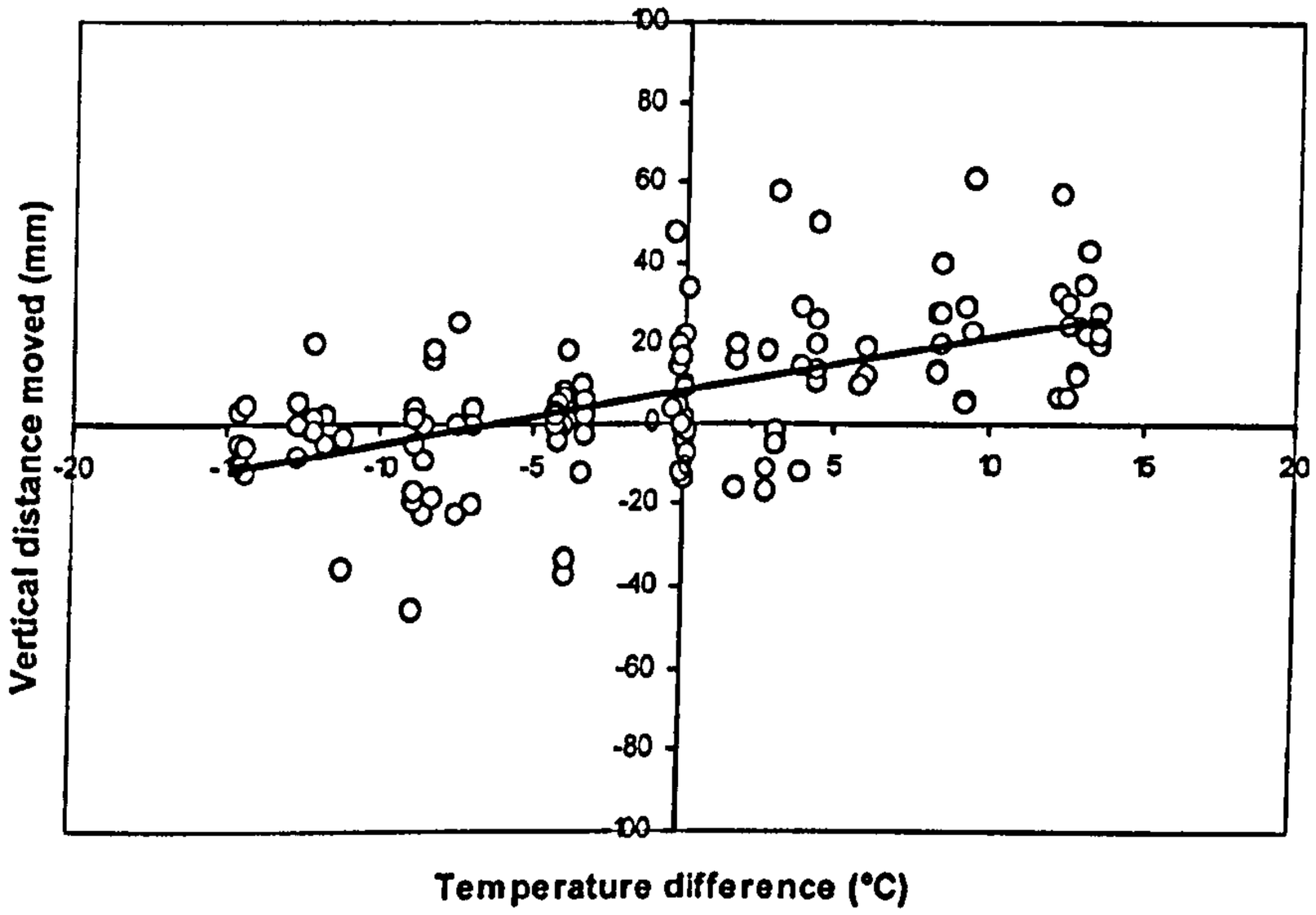

Figure 3. Vertical distance moved by *P. ovis* mites in the presence of a temperature gradient but in the absence of any unidirectional light gradient ($y = 1.3283x + 8.0165$, $r^2 = 0.3271$).

between temperature difference and vertical distance travelled ($F = 30.98$, $P < 0.001$, $r^2 = 36.25\%$), but the mites did not move downwards so far when the gradient was hotter at the top. Similarly, when illumination was from below only, the movement of the mites was displaced upwards (Figure 4b). In this case there was a weak, but significant, polynomial relationship between temperature difference and vertical distance moved ($F_{95} = 5.24$, $p = 0.007$, $r^2 = 10.1\%$), but in this case the movement upwards was least when the temperature gradient was smallest.

Discussion

The results of the present study showed that adult female *P. ovis* mites responded positively to temperature and negatively to gravity and light. Mites moved towards the hotter end of a temperature gradient, regardless of whether this was above or below. Similar responses are well known in ticks. For example, *I. ricinus* larvae and nymphs are attracted to a warmed tube (Lees 1948); a positive thermotaxis is usually interpreted as behaviour that would increase the probability of attaching to a host animal.

A negative response to light has also been observed previously in mites, for example the Banks grass mite *Oligonychus pratensis* (Banks) (Acari: Tetranychidae) (Li and Margolies 1991). In the present study the high degree of sensitivity to light shown by *Psoroptes* is particularly interesting since no organs

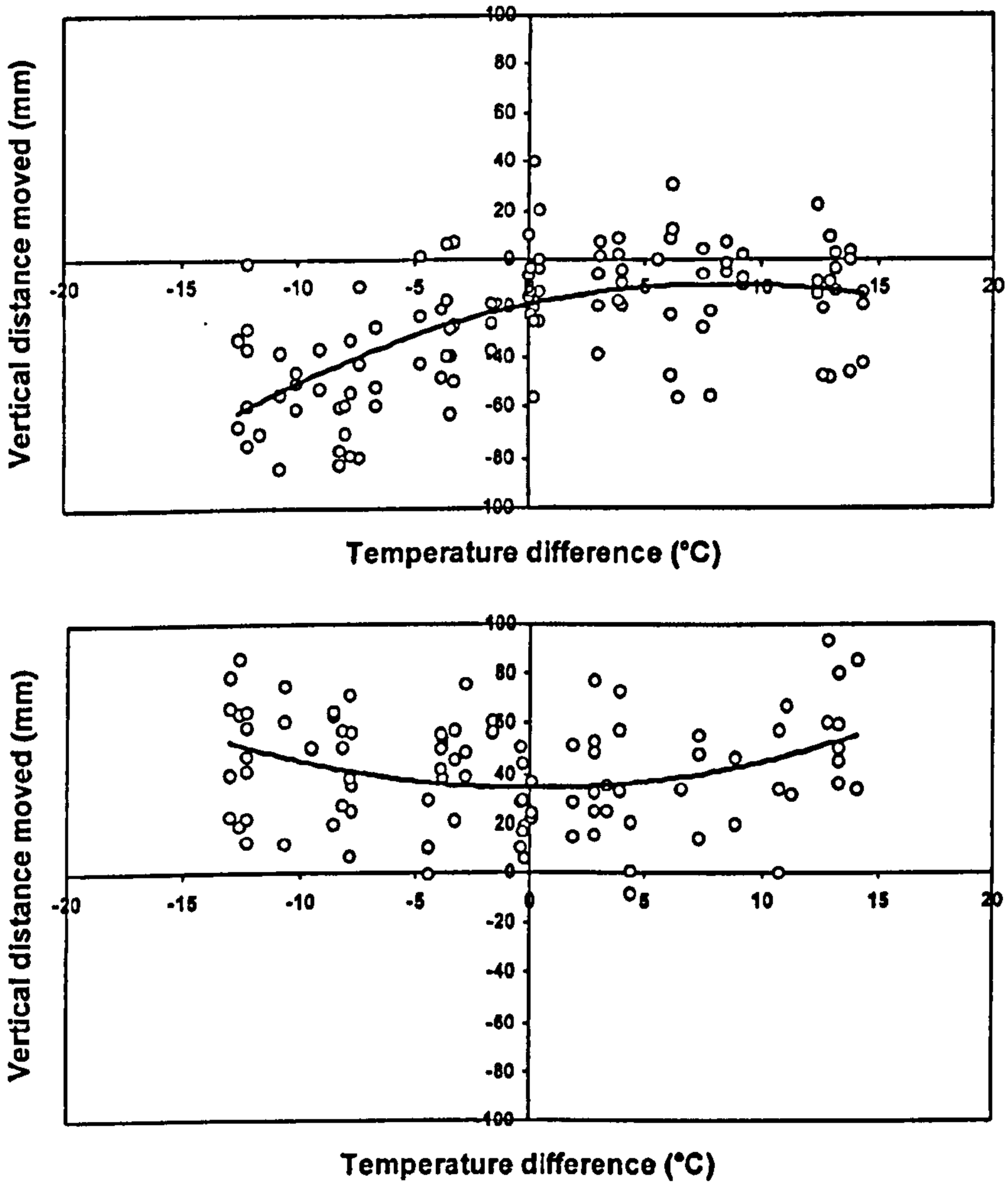

Figure 4. Vertical distance moved by *P. ovis* mites in the presence of a temperature gradient and a unidirectional light gradient from (a) above ($y = -0.1185x^2 + 1.995x$-18.256, $r^2 = 36.2\%$) and (b) below ($y = 0.1073x^2 - 0.0241x + 34.282$, $r^2 = 10.1\%$).

capable of light detection have yet been identified. The tick, *Hyalomma truncatum* (Koch) shows positive scototaxis towards stationary, two-dimensional objects (Kopp and Gothe 1995). The time taken for locomotion towards the object to begin is shortened in the presence of a carbon dioxide gradient (Kopp and Gothe 1995). Although this scototaxis may be seen as host-seeking behaviour, it was interpreted by these authors as a response to allow protection from harsh climatic conditions, as stationary objects were preferred to moving ones and the presence of a temperature gradient, mimicking the proximity of a host, did not enhance the response. This

suggests that in addition to aiding host-seeking, the various cues used by mites and ticks may also promote off-host survival. In the present study, since desiccation is an important mortality factor for *Psoroptes* mites off-host (Smith et al. 1999), movement away from light may also help mites move to locations with high relative humidity, thereby maximising off-host survival time.

For *Psoroptes*, the movement to areas of lower light intensity and higher temperature might be expected to help them to maintain their position on a host animal or help them locate the skin surface of a new host when displaced into the environment. In sheep, *Psoroptes* mites are found on the skin at the base of the fleece, an area where light intensity will be low and temperature will be between 30 and 40 °C (Wall et al. 1992). Light cues may increase the rate at which mites move to the base of the fleece thus allowing more rapid establishment and a greater success of transmission. In the present study, changes in activity with light or temperature gradients were continuous; at no point were step-changes in orientation or rate of movement observed. This suggests a graduated response to environmental cues is used to move *P. ovis* to areas of favourable conditions. A similar mechanism has been suggested by Li and Margolies (1991) in the location of the Banks grass mite on its host plant.

Gravitational cues are often used to ensure successful transmission to a new host in many tick species and questing stages climb the vegetation to increase their probability of contact with a potential host. Further to this negative geotaxis, tick larvae have been shown to be able to detect their height above ground and form clumps at 50–190 cm above ground level in the absence of external cues, even if the vegetation height is greater than this (McPherson et al. 2000). The level at which the larvae clump corresponds to the torso heights of their hosts therefore increasing the chance of transmission. A similar response is also seen in phyophagous species, for example the black-currant gall mite *Cecidophyopsis ribis* (Westwood) (Acari: Eriophyidae) shows a negative response to gravity (Herr 1991). A climbing response was seen in the present study and such behaviour might be expected to bring off-host mites into positions where they would be more likely to make contact with a new host. However, the climbing response was largely abolished by the presence of strong illumination from above. The functional significance of this behaviour in *Psoroptes* is uncertain, but since these mites are highly susceptible to desiccation (Smith et al. 1999), it may be that climbing and exposure to sunlight is only attempted in darkness or conditions of low light intensity when humidity is likely to be relatively high.

The data presented here support the suggestion that there may be a hierarchy among stimuli used by parasites to find a new host (MacInnis 1976). The light cues modified the temperature-gradient information and both effectively abolished the climbing response. MacInnis also suggested that there may be synergistic effects among stimuli and this was also observed here; when exposed to gradients of both temperature and light, mites moved greater distances than when either cue was present alone. In addition to helping understand the behaviour of *Psoroptes* mites both on and off the host animal, an understanding of the responses of mites to environmental cues is essential in attempts to establish *in vitro* colonies of this species.

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References

Anderson R.B., Scrimgeour G.J. and Kaufman W.R. 1998. Responses of the tick *Amblyomma hebraeum* (Acari: Ixodidae), to carbon dioxide. Exp. Appl. Acarol. 22: 667–681.

Bates P.G. 1999. Inter- and intra-specific variation within the genus *Psoroptes* (Acari: Psoroptidae). Vet. Parisitol. 83: 201–217.

Berriatua E., French N.P., Wall R., Smith K.E. and Morgan K.L. 1999. Within-flock transmission of sheep scab in naive sheep housed with single infested sheep. Vet. Parasitol. 83: 277–289.

Berriatua E., French N.P., Broster C.E., Morgan K.L. and Wall R. 2001. Effect of infestation with *Psoroptes ovis* on the nocturnal rubbing and lying behaviour of housed sheep. Appl. Anim. Behav. Sci. 71: 43–55.

Carroll J.F., Klun J.A. and Schmidtmann E.T. 1995. Evidence for kairomonal influence on selection of host-ambushing sites by adult *Ixodes scapularis* (Acari: Ixodidae). J. Med. Entomol. 32: 119–125.

Corke M.J. and Broom D.M. 2000. Sheep scab: a questionnaire survey of sheep farmers. Proc. Sheep Vet. Soc. 24: 115–121.

Evans L.M., Dawson D.A., Wall R., Burke T. and Stevens J.R. 2003. Isolation of *Psoroptes* scab mite microsatellite markers (Acari: Psoroptidae). Mol. Ecol. Notes 3: 420–424.

Falconi F., Ochs H. and Deplazes P. 2002. Serological cross-sectional survey of psoroptic sheep scab in Switzerland. Vet. Parasitol. 109: 119–127.

French N.P., Berriatua E., Wall R., Smith K. and Morgan K.L. 1999. Sheep scab outbreaks in Great Britain between 1973 and 1992: spatial and temporal patterns. Vet. Parasitol. 83: 187–200.

Herr R. 1991. Investigations on resistance of ribes against the black-currant gall mite *Cecidophyopsis ribis* (Westw.) (Acari, Eriophyidae). Bioassays in the laboratory and infestation experiments in the field. J. Appl. Entomol. 112: 181–193.

Kopp K. and Gothe R. 1995. *Hyalomma truncatum* (Acari: Ixodidae) – investigations into the scototaxis of unfed adult ticks. Exp. Appl. Acarol. 19(1): 53–64.

Lees A.D. 1948. The sensory physiology of the sheep tick *Ixodes* ricinus. J. Exp. Biol. 25: 145.

Li J.B. and Margolies D.C. 1991. Factors affecting location of banks grass mite *Oligonychus pratensis* (Acari: Tetranychidae) on corn leaves. Exp. Appl. Acarol. 12: 27–34.

MacInnis A.J. 1976. How parasites find hosts: some thoughts on the inception of host-parasite integration. In: Kennedy C.R. (ed) Ecological Aspects of Parasitiology. North-Holland Publishing Co., Amsterdam, The Netherlands, pp. 3–20.

Mathieson B.R.F. 1995. An investigation of *Psoroptes ovis*, the sheep scab mite: with a view to developing an *in vitro* feeding system. Ph.D. Thesis, University of Wales, UK.

Mathieson B.R.F. and Lehane M.J. 1996. Isolation of the Gram-negative bacterium, *Serratia marcescens*, from the sheep scab mite, *Psoroptes ovis*. Vet. Rec. 138: 210–211.

McMahon C. and Guerin P.M. 2002. Attraction of the tropical bont tick, *Amblyomma variegatum*, to human breath and to the breath components acetone, NO and CO_2. Naturwissenschaften 89: 311–315.

McPherson M., Shostak A.W. and Samuel W.M. 2000. Climbing simulated vegetation to heights of ungulate hosts by larvae of *Dermacentor albipictus* (Acari: Ixodidae). J. Med. Entomol. 37: 114–120.

Meintjies T., Fourie L.J. and Horak I.G. 2002. On-host ecology and off-host survival of the sheep scab mite *Psoroptes ovis*. Onderstepoort J. Vet. Res. 69: 273–283.

O'Brien D.J., Gray J.S. and O'Reilly P.F. 1994. Survival and retention of infectivity of the mite *Psoroptes ovis* off the host. Vet. Res. Commun. 18: 27–36.

Sanders A., Froggatt P., Wall R. and Smith K.E. 2000. Life-cycle stage morphology of *Psoroptes* mange mites. Med. Vet. Entomol. 14(2): 131–141.

Sinclair A.N. and Kirkwood A.G. 1983. Feeding behaviour of *Psoroptes ovis*. Vet. Rec. 15: 65.

Smith K.E., Wall R., Berriatua E. and French N.P. 1999. The effects of temperature and humidity on the off-host survival of *Psoroptes ovis* and *Psoroptes cuniculi*. Vet. Parasitol. 83: 265–275.

Sweatman G.K. 1958. On the life history and validity of the species in Psoroptes, a genus of mange mites. Can. J. Zool. 36: 905–929.

Wall R., French N.P. and Morgan K.L. 1992. Effects of temperature on the development and abundance of the sheep blowfly *Lucilia sericata* (Diptera: Calliphoridae). Bull. Entomol. Res. 82: 125–131.

Wilson G.I., Blachut K. and Roberts I.H. 1977. The infectivity of scabies (mange) mites, *Psoroptes ovis* (Acarina: Psoroptidae), to sheep in naturally contaminated enclosures. Res. Vet. Sci. 22: 292–297.

Zahler M., Essig A., Gothe R. and Rinder H. 1998. Genetic evidence suggests that *Psoroptes* isolates of different phenotypes, hosts and geographic origins are conspecific. Int. J. Parasitol. 28: 1713–1719.

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Morphological and molecular comparison of host-derived populations of parasitic *Psoroptes* mites

K. R. PEGLER[1], L. EVANS[2], J. R. STEVENS[2] and R. WALL[1]
[1]School of Biological Sciences, University of Bristol, U.K. and [2]School of Biosciences, University of Exeter, U.K.

Abstract. Infestation by parasitic *Psoroptes* mites (Acari: Psoroptidae) is an important cause of economic loss and welfare problems in livestock in many areas of the world. At least five species within this genus have been recognized, based on the host infested, the infestation site and differences in length of the opisthosomal setae of adult male mites. Here the integrity of these species is considered by subjecting populations of mites from a range of host species and geographical locations to simultaneous morphological and molecular genetic analyses. Morphological analysis showed that there were significant differences in shape and size between mite populations from different hosts, and that length of the outer opisthosomal setae in males and the homologous seta in females were the most important distinguishing character in adults. However, considerable variation in outer opisthosomal seta length was evident within and between populations of mites, and differences were not clearly related to host-species or geographical origin and did not support the accepted species differences. Molecular characterization using sequence data from the mitochondrial second internal transcribed spacer (ITS-2) region and microsatellite markers found little or no consistent host-related variation between the mite population samples. The results suggest that there is no case for considering the *Psoroptes* mites from the different hosts examined as separate species and that the morphological variation observed therefore may represent phenotypic adaptation to the local microenvironment on particular species of host.

Key words. *Psoroptes*, mange, microsatellites, mites, morphology, second internal transcribed spacer region, species integrity.

Introduction

Psoroptes mites are superficial skin parasites of a range of mammalian hosts. Mites are believed to feed on a lipid emulsion of skin cells, bacteria and lymph, produced on the host skin as a result of a hypersensitivity reaction to the presence of antigenic mite faecal material (Blake *et al.*, 1978; Sinclair & Kirkwood, 1983; van den Broek *et al.*, 2003) Infestation may be chronic or even subclinical and localized, often in the ear of the host, or it may be acute and more generalized over the entire body, when it is described as psoroptic mange (Bates, 1999). Psoroptic mange is an important disease of livestock, particularly sheep, worldwide (van den Broek & Huntley, 2003; Colebrook & Wall, 2004), resulting in severe irritation to the host, making it restless and scratch at the infested areas (Corke & Broom, 1999; Berriatua *et al.*, 2001; Bisdorff *et al.*, 2005). This self-trauma results in hair loss, skin damage and weight loss and, if left untreated, can lead to death due to dehydration, pneumonia or bacterial septicaemia (Roberts *et al.*, 1971; Tarry, 1974).

The genus *Psoroptes* is distinguished by the presence of a terminal sucker on a relatively long, jointed pre-tarsus (Hirst, 1922; Babcock & Black, 1933; Sweatman, 1958; Sanders *et al.*, 2000). Following the initial description by Hering (1838), up to nine species of *Psoroptes* were proposed, each distinguished from the others mainly by the different mammalian hosts they infested, with varietal names such as *ovis* or *bovis* appended, depending on the

Correspondence: Professor Richard Wall, School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG. Tel./Fax: +44 (0)117 9289182; e-mail: richard.wall@bristol.ac.uk

2 host species from which they were obtained (Raillet, 1893; Stockman & Berry, 1913; Shilston, 1915). In 1958, Sweatman carried out a detailed study in an attempt to find a stable morphological character which could be used to separate putative species of the genus *Psoroptes* (Sweatman 1958). He proposed that the outer opisthosomal setae of adult males could be used to distinguish five true species. These included *Psoroptes cuniculi* and *P. cervinus* from the ears of rabbits and bighorn sheep, respectively, and *P. equi*, *P. bovis* and *P. natalensis* which are described as primarily body mites of horses, cattle and buffalo, respectively.

This classification has achieved general acceptance in the literature (Strong & Halliday, 1992). However, use of this character has proved difficult. A high degree of variance in setae lengths within populations from any individual host and populations from different hosts have been reported (Lange *et al.*, 1980; Wright *et al.*, 1984; Bates & Sayers, 2002). The concept of host-specific species of *Psoroptes* has also been challenged by cross-host transmission studies, which have successfully transferred mites between rabbits, goats, sheep and cattle (Meleney, 1967; Wright, 1982). Successful cross-mating of *P. ovis* and *P. cuniculi* in the ears of rabbits to produce viable offspring has been claimed (Wright *et al.*, 1983); however, to date this latter study has not been successfully repeated or confirmed.

Boyce & Brown (1991) found only minor antigenic differences between mites from cattle, rabbits and bighorn sheep, and found mites from mule deer to be antigenically identical to those from bighorn sheep. Host transfer experiments of mites between rabbits and sheep have also shown that these mites are antigenically cross-reactive (Siegfried *et al.*, 2004). A number of molecular studies have generated sequence data for the second internal transcribed spacer region (ITS-2) of the ribosomal DNA. This rDNA sequence lies between the regions coding for the 5.8S and 28S ribosomal subunits and has been extensively used in species status studies in related groups of mites and ticks (Zahler *et al.*, 1995; Essig *et al.*, 1999; Lohse *et al.*, 2002). However, using this sequence, only low levels of variation in samples of *Psoroptes* from a variety of host species have been reported, with as little as a single base difference observed (Zahler *et al.*, 1998; Ochs *et al.*, 1999). Interestingly, however, opposite conclusions have been drawn from this similar pattern of variation, with one group believing that the presence of any variation supports separate species status (Ochs *et al.*, 1999), whereas the other group concluded that the variation was insufficient to separate putative species (Zahler *et al.*, 1998). A study of the first internal transcribed spacer (ITS-1) in *Psoroptes* mites from the United States found similarly minimal levels of variation between samples (Ramey *et al.*, 2000), which again bore no relationship to putative mite species.

The question of whether the genus *Psoroptes* is composed of one or several host-specific species has important practical epidemiological implications for the potential for cross-transmission and control of this disease. The aim of the work presented here therefore was to undertake a detailed re-examination of the morphology of *Psoroptes* mites derived from a range of hosts and to complement this with a simultaneous high resolution molecular analysis of specimens from the same samples, using a combination of ITS-2 sequence analysis and *Psoroptes*-specific microsatellite markers.

Materials and methods

Morphological analysis

Samples of *Psoroptes* mites were collected, or were provided, from various host species and from various geographical locations worldwide (Table 1). Adult mites were cleared in Nesbitt's fluid (chloral hydrate 40 g, distilled water 25 mL, HCl conc. 2.5 mL) for approximately 3 days before mounting, dorsal side uppermost, in Hoyer's medium (chloral hydrate 200 g, distilled water 50 mL, gum arabic 30 g, glycerine 20 mL). The preparation was then dried at approximately 25°C for 3 days and the edge of the glass coverslip sealed with clear nail varnish.

Specimens were examined under a binocular light microscope and digital photographs and measurement of morphological features were captured by computer (Qwin, Leica Imaging Systems Ltd, Cambridge, U.K.). Five morphological features in males and six in females were found in preliminary assessments to be informative and not highly correlated with each other. For males these were: outer opisthosomal seta length, width of body at base of leg III, gnathosoma length, length of ambulacrum of leg I and propodosomal seta length. In females these were: width of body at base of leg III, gnathosoma length, length of ambulacrum of leg I, propodosomal seta length (pair below vulva), leg III posterior seta length and outer opisthosomal seta length. The outer opisthosomal setae in female mites were the outermost of the four setae found at the posterior of the ventral opisthosoma and were considered to be homologous to those found in adult males. Although no significant differences between left and right-hand measurements of paired characters were detected, both sides were nevertheless measured and the mean value used for analysis.

Discriminant analysis (SPSS 12.0, SPSS Inc. Chicago, U.S.A.), with host species as the grouping variable, was used to determine linear combinations of morphological characters that could be used to distinguish between mites collected from the various host species. This analysis was carried out for both adult male and female mites, first by pooling samples from the same species of host and then separately for samples from each individual host. For characters identified by the discriminant analysis as most highly informative, normality was confirmed using a 1-sample K-S-test and homogeneity of variances by Levene's test. Differences between character lengths in the samples obtained from different hosts were then considered using one-way ANOVA and Tukey HSD multiple range tests.

Table 1. Host, origin and, where known, site on the host from where the sample was obtained. The table also indicates which samples were included in the molecular and morphological analyses and, when included in the morphological analysis, in parenthesis the number of male and female mites examined (+, included; –, not included). ITS2, second internal transcribed spacer.

			Molecular analysis		Morphological analysis	
Host species	Origin	Site	ITS2	Microsatellites	No. males	No. females
Domestic rabbit	Bristol, U.K. (University of Bristol isolate)	Ear	+	+	+ (48)	+ (45)
	Bristol U.K. (Veterinary surgery)	Ear	+	+	+ (9)	+ (4)
	Argentina	Ear	+	+	–	–
Sheep	Yorkshire, U.K.	Body	+	+	+ (9)	+ (10)
	Scotland, U.K.	Body	+	+	+ (3)	+ (5)
	Gloucestershire, U.K.	Body	+	+	+ (2)	–
	Scotland, U.K.	Body	+	+	+ (6)	–
	Lancashire, U.K.	Body	+	+	+ (6)	+ (3)
	Wales, U.K.	Body	+	+	–	+ (12)
	Lancashire × Cornwall, U.K. (VLA Strain)	Body	–	+	+ (3)	+ (10)
	York, U.K.	Body	+	+	–	+ (5)
	Devon, U.K.	Body	+	+	–	+ (1)
	Cornwall, U.K.	Body	+	+	+ (4)	+ (3)
	Cumbria, U.K.	Body	+	+	–	+ (1)
	Scotland, U.K.	Body	–	+	+ (1)	+ (3)
	Yorkshire, U.K.	Body	–	+	+ (2)	+ (1)
	Dublin, Ireland (1988 strain – isolated since 1986)	Body	+	+	+ (1)	+ (1)
	Chester, U.K.	Body	–	–	+ (1)	+ (6)
	Wales, U.K.	Body	–	–	–	+ (5)
	Powys, Wales, U.K.	Body	–	–	+ (1)	+ (4)
	Gwynedd, Wales, U.K.	Body	–	–	+ (1)	+ (3)
	Powys, Wales, U.K.	Body	–	–	–	+ (1)
	Wales, U.K.	Body	–	–	+ (1)	+ (6)
	SW England, U.K.	Body	–	–	+ (3)	+ (4)
	Bloemfontein, South Africa	Body	–	–	+ (8)	+ (18)
	Northumberland, U.K.	Body	+	+	–	–
	Argentina	Body	–	+	–	–
Alpaca	Chile, South America	Ear	+	+	+ (1)	+ (1)
	Animal imported into U.K. from Chile; source of infestation unknown.	Ear	–	+	+ (8)	+ (7)
	Chile, South America	Ear	+	+	–	–
Goat	Bristol, U.K. (VLA)	Ear	–	–	+ (2)	+ (1)
	Georgia, U.S.A.	Not known	–	+	+ (6)	–
Cow	Texas, U.S.A.	Not known	–	+	+ (14)	+ (18)
	Belgium	Body	–	–	+ (7)	+ (29)
Big horn sheep	New Mexico, U.S.A.	Not known	–	+	+ (7)	+ (6)
	New Mexico, U.S.A.	Not known	–	+	+ (5)	+ (8)
	Utah, U.S.A.	Not known	–	+	+ (1)	–
	North America	Not known	–	+	–	–
Mule deer	New Mexico, U.S.A.	Not known	+	+	+ (6)	+ (6)
	New Mexico, U.S.A.	Not known	+	+	–	–
	New Mexico, U.S.A.	Not known	–	+	–	–
	New Mexico, U.S.A.	Not known	–	+	–	–
Elk	Idaho, U.S.A.	Not known	–	+	+ (6)	+ (1)
White tailed deer	U.S.A.	Not known	–	+	+ (5)	+ (5)

Second internal transcribed spacer region fragment amplification and sequencing

Prior to analysis, mite samples were stored in 100% ethanol at 5°C. DNA was extracted from individual mites by homogenizing the whole body in 40 μL of 50 mM NaOH in individual 1.5-mL microfuge tubes using a domestic power drill attached to a 'Pellet Pestle' (Kimble Kontes Inc, New Jersey, U.S.A.). The homogenate was incubated at 95°C for 15 min before neutralizing with 6 μL of 1 M Tris-HCl (pH 8.0). The identity of mite samples used for these molecular analyses is detailed in Table 1. Two pairs of primers were used amplifying different length sections of the ITS-2 region, Rib-4 and Rib-3 (Zahler *et al.*, 1998) and Huo-3 and Huo-4 (Ochs *et al.*, 1999).

Polymerase chain reaction (PCR) was carried out in a Thermo Hybaid Express™ thermal cycler in 15-μL volumes containing 0.75 μL of whole *Psoroptes* mite homogenate, 500 nM of each primer, 0.25 U of AGS*Gold*™ DNA polymerase (Hybaid, Ashford, U.K.) in the manufacturer's buffer [75 mM Tris/HCl (pH 9.0), 20 mM $(NH4)_2SO_4$, 0.01% Tween-20], with 200 μM of each dNTP and 1.6 mM $MgCl_2$. The reaction profile was 94°C for 3 min, then 35 cycles of 94°C for 30 s, T_a°C for 60 s and 72°C for 60 s, and finally 72°C for 5 min to allow extension to complete. T_a°C was the tested optimal temperature for each pair of primers and found to be 59°C for Rib-3 and Rib-4 and 64°C for Huo-3 and Huo-4. Following some difficulty in amplifying directly from homogenized mite extract, bovine serum albumin (fraction V) and Tween 20 were also added to the PCR at a final concentration of 1 mg/mL and 1%, respectively. The primer pair that produced the longest sequence read, Rib-3 and Rib-4, was used to generate sequence for as many samples as possible; however, some samples proved difficult to sequence and hence a smaller product was generated using the Huo-3 and Huo-4 primers. It was possible to achieve a shorter but stronger amplification with this arrangement so that these difficult samples could be included, albeit with a shorter sequence.

PCR products were purified from agarose gel using the MinElute™. Gel Extraction Kit (Qiagen, Crawley, U.K.), and sequenced (Lark Technologies Inc, Essex, U.K.) in both the 5′ and 3′ direction allowing the production of consensus sequences.

These consensus sequences were put through a BLASTN search (Altschul *et al.*, 1997) to check their identity against other sequences registered in the database. Two published ITS-2 sequences from *P. ovis* (EMBL accession numbers AF123079 and AF123080, Ochs *et al.* 1999) were included as positive controls to confirm the status of the new sequences as genuine *Psoroptes* ITS-2 sequence, and one from *Otodectes cynotis* (EMBL accession number AF367703) was included as an outgroup.

Sequences were then aligned using ClustalX (Thompson *et al.*, 1997), prior to performing maximum parsimony analysis using PAUP* 4.0b10 (Swofford, 2003). Parsimony analysis of the 26 mite ITS-2 sequences (Table 1) was performed with 100 bootstrap replicates. The default options of PAUP were used: heuristic search strategy, TBR branch swapping, zero length branches collapsed and 10 random addition sequences (bootstrap analyses used simple addition).

Microsatellite analysis

The same selection of *P. ovis* samples were PCR amplified with a suite of nine species-specific microsatellite markers (Evans *et al.*, 2003). The individuals genotyped in this way are detailed in Table 1. Amplification was carried out under the same conditions as the ITS-2 reactions using the same mite homogenates. PCR products were visualized on 6% polyacrylamide gels stained with silver (Promega, Southampton, U.K., Bassam *et al.* 1991). Allele sizes were identified by comparison to a 10 bp DNA ladder, 25 bp DNA ladder (both Invitrogen, Paisley, U.K.) and ϕX174 RF DNA/*Hae*III DNA marker (Abgene, Epsom, U.K.). The product sizes produced by this amplification were treated as independent characters and, as previously, were subjected to bootstrapped maximum parsimony analysis using PAUP*4.0b10 (Swofford, 2003).

Results

Morphology: adult males

Discriminant analysis, with host species as the grouping variable, identified five significant linear functions. Function 1, with an eigenvalue of 4.03, was most highly correlated with outer opisthosomal seta length with a correlation coefficient of 0.95. Function 1 alone explained 74.3% of the total variance and the first three functions combined explained 96.2% of the variance. Functions 2 and 3 were most highly correlated with body width and gnathosoma length and explained 12% and 9.9% of the variance, respectively. A plot of function 1 against 2 allows mites to be distinguished morphologically by host (Fig. 1). As outer opisthosomal length was found to be the main discriminatory feature, only this character will be considered in further analysis.

The difference between the outer opisthosomal seta lengths of adult male mites from different host species was highly significant ($F_{8,168} = 76.5$, $P < 0.001$), with the setae of mites from cattle being the longest and those from rabbits the shortest (Fig. 2). Very little variation was evident between the outer opisthosomal seta lengths of mites from elk, bighorn sheep, alpaca, mule deer, white-tailed deer, sheep and goat. The outer opisthosomal seta lengths of mites from rabbits were significantly shorter than the mites of all hosts other than goats. The outer opisthosomal seta lengths of mites from cattle were significantly longer than those of mites from all other host species.

When the outer opisthosomal seta lengths were compared for individual samples, a relatively large amount of variation between mites from the same host species was seen, making it difficult to assign mites to a particular host species based solely on their seta lengths (Fig. 3). For example, there was a significant difference between the two cattle isolates from U.S.A. and Belgium ($t_{19} = -4.23$, $P < 0.001$) and between the two rabbit isolates, both from Bristol ($t_{55} = 5.97$, $P < 0.001$). There was also a significant difference between outer opisthosomal seta lengths of males mites from different sheep isolates ($F_{15,36} = 3.10$, $P = 0.003$).

Morphology: adult females

Discriminant analysis, using host species as the grouping variable (excluding goat and elk samples, each of

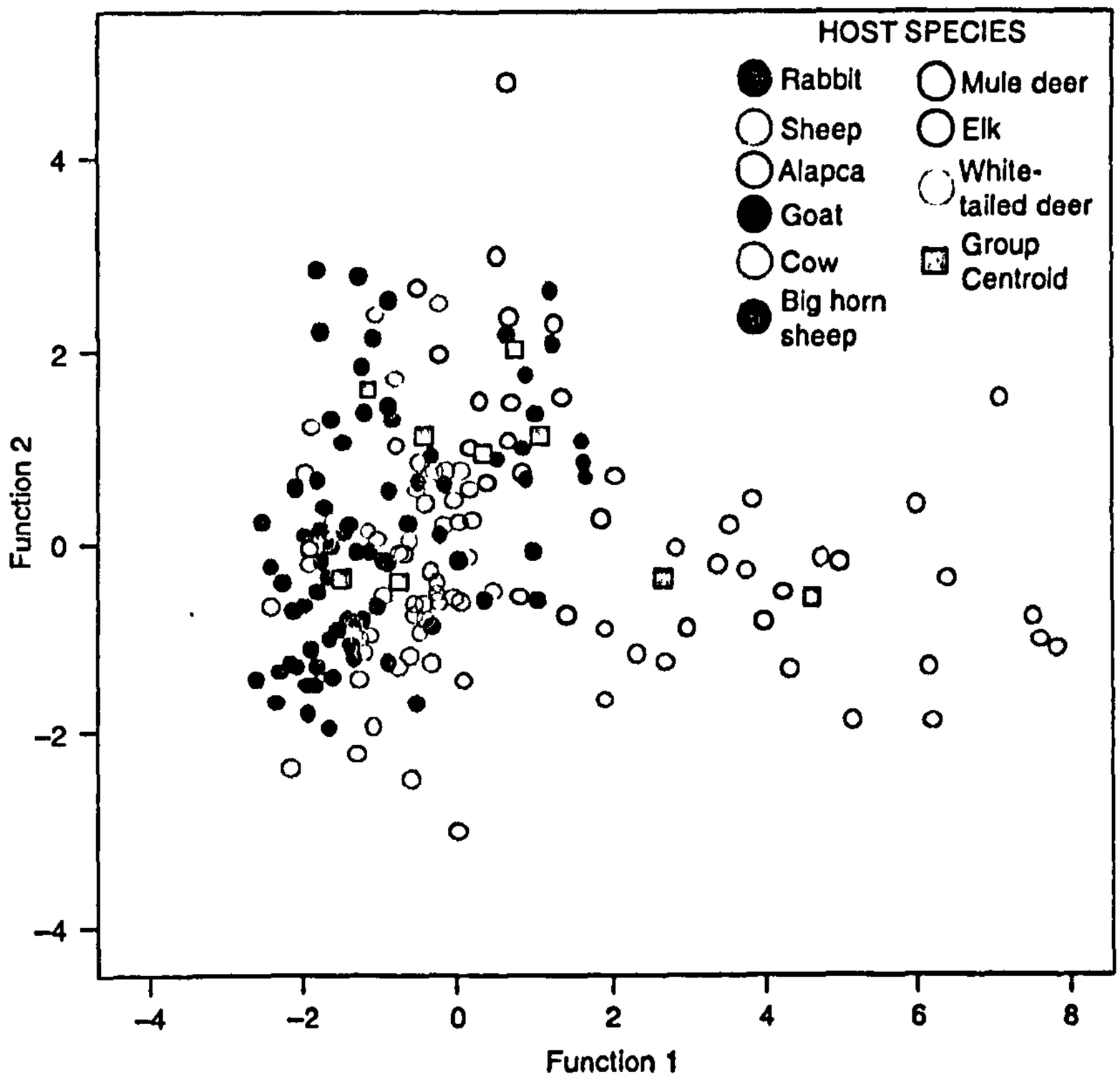

Fig. 1. Two linear functions of five morphological characters measured on 177 adult male mites as revealed by discriminant analysis. Functions 1 and 2 are most highly correlated with outer opisthosomal seta length and body width, respectively.

which included only one female mite), identified four significant functions. Function 1 had an eigenvalue of 2.04 and, as for males, was most highly correlated with outer opisthosomal seta length with a correlation of 0.82 ($r^2 = 76.6\%$). Functions 2 and 3 were most highly correlated with gnathosoma length and leg 1 ambulacrum

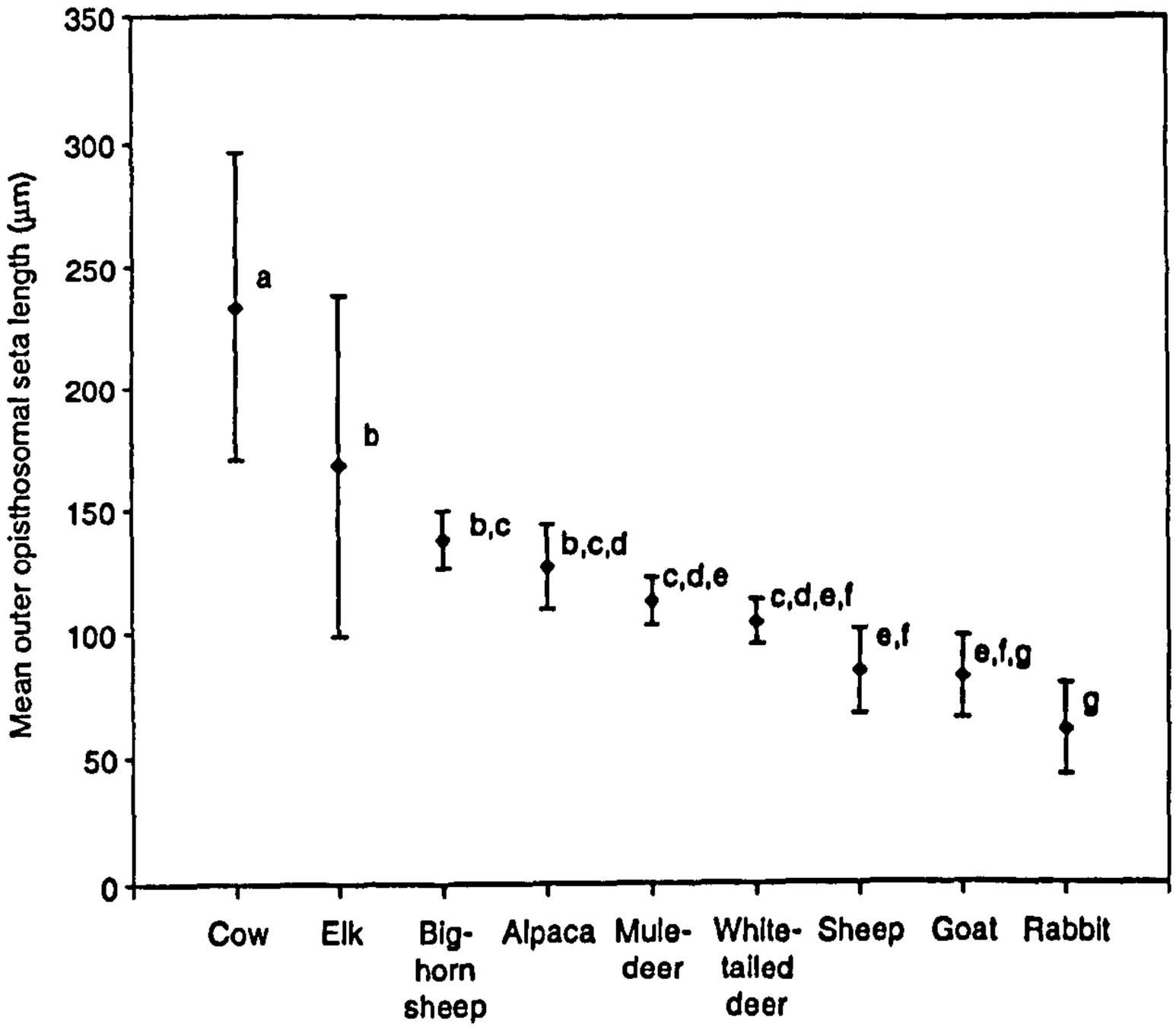

Fig. 2. Mean outer opisthosomal seta length (µm ± SD) of adult male *Psoroptes* mites collected from a range of host species. Letters indicate points between which there is no statistically significant difference ($F_{8,168} = 76.46$, $P < 0.001$).

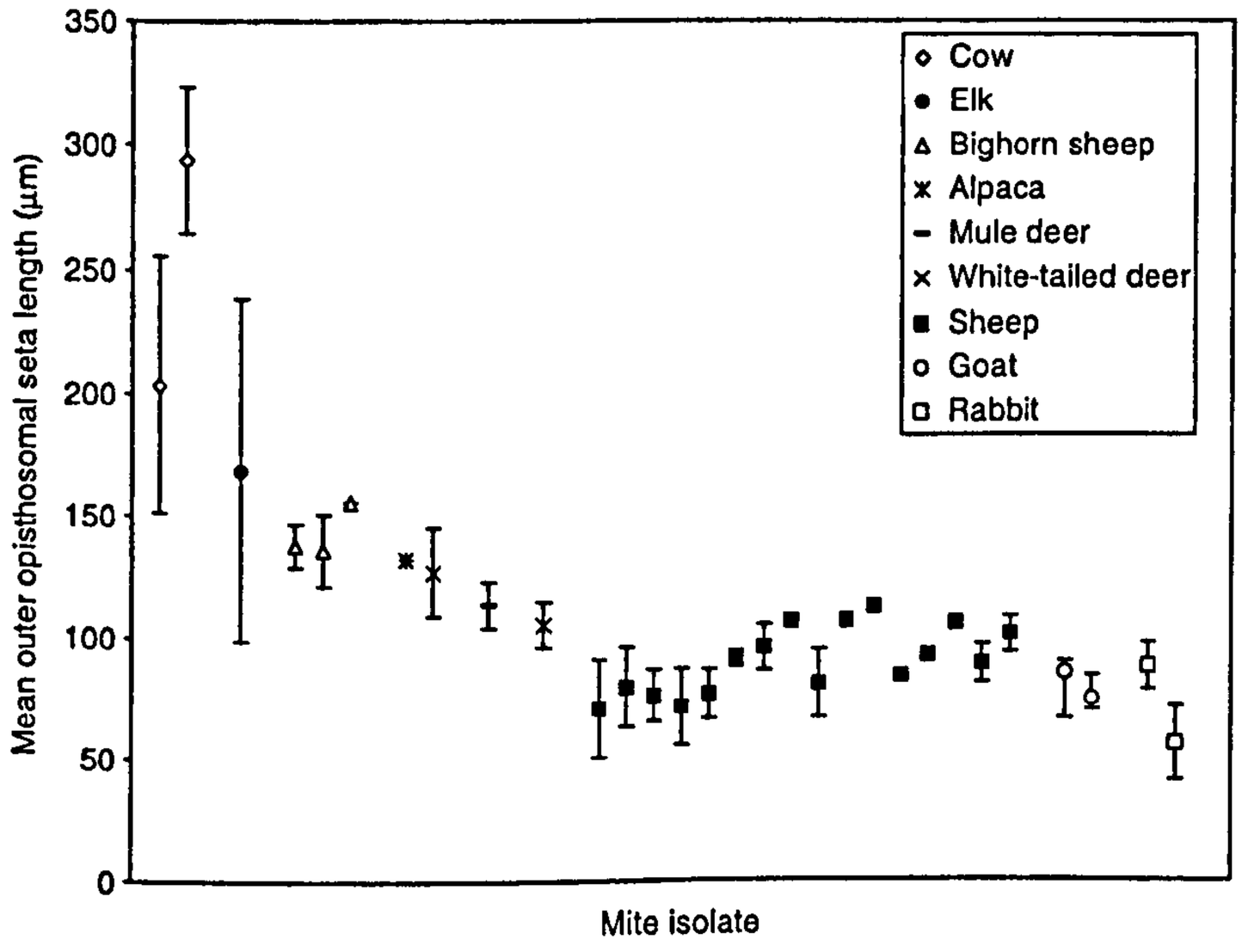

Fig. 3. Mean outer opisthosomal seta length (µm ± SD) of adult male *Psoroptes* mites of all isolates examined.

length, respectively, and functions 1 to 3 together accounted for 95.0% of the total variance. When functions 1 and 2 were plotted against each other, some separation of mites from different host species into groups was seen though there is considerable variation (Fig. 4). As outer opisthosomal length was found to be the main discriminatory feature, only this character will be considered in further analysis.

Comparison of the adult female outer opisthosomal seta lengths by host species showed that there is a significant difference ($F_{6,224} = 50.9$, $P < 0.001$). Outer opisthosomal setae were again longest in mites from cattle and shortest in mites from rabbits (Fig. 5). However there was no difference between the outer opisthosomal seta lengths of mites from alpaca, white-tailed deer, sheep, big-horn sheep or mule deer. There was no significant difference between

Colour Figure

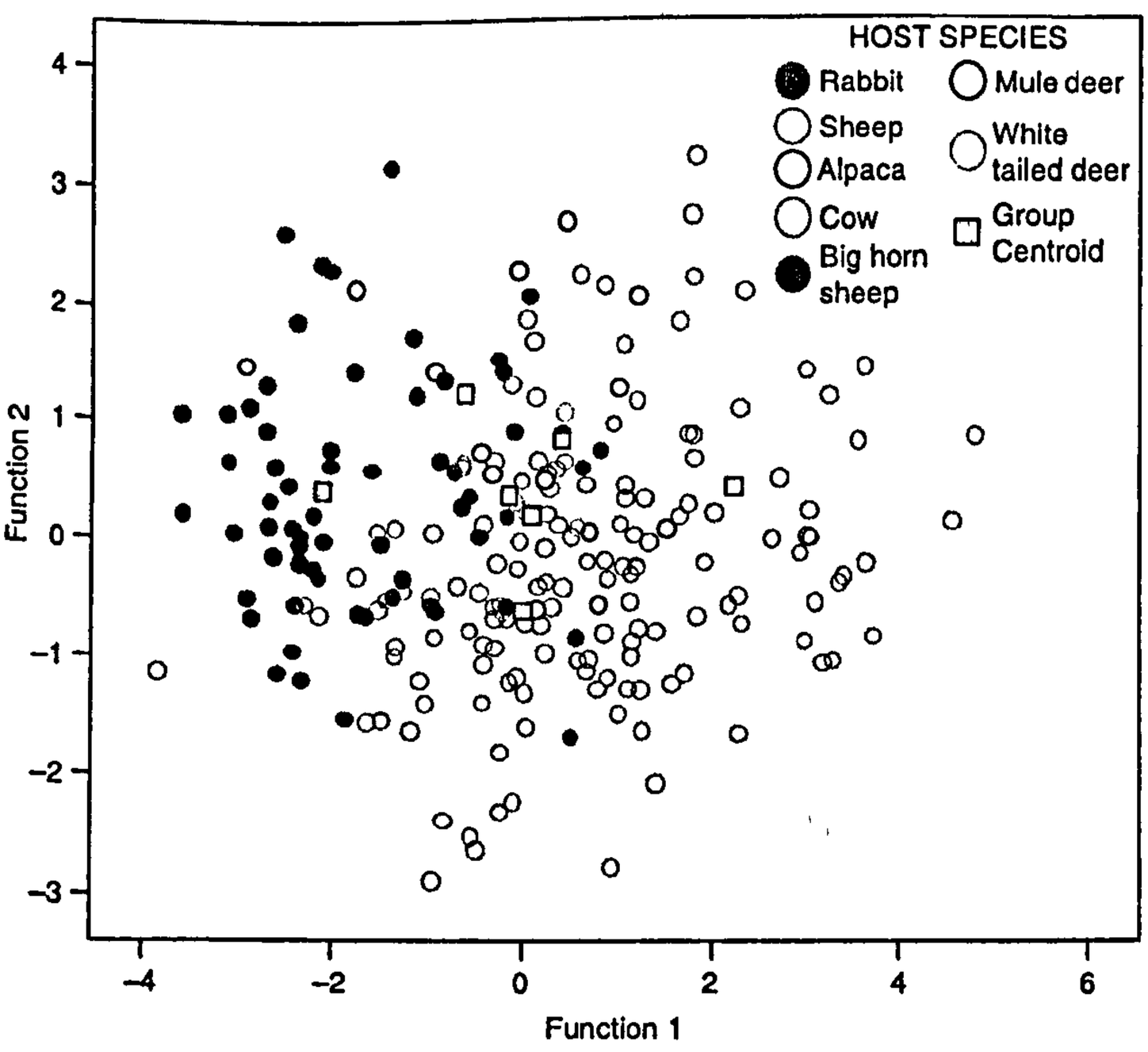

Fig. 4. Two linear functions of six morphological characters measured on 231 adult female mites as revealed by discriminant analysis. Functions 1 and 2 are most highly correlated with outer opsithosomal seta length and gnathosoma length, respectively.

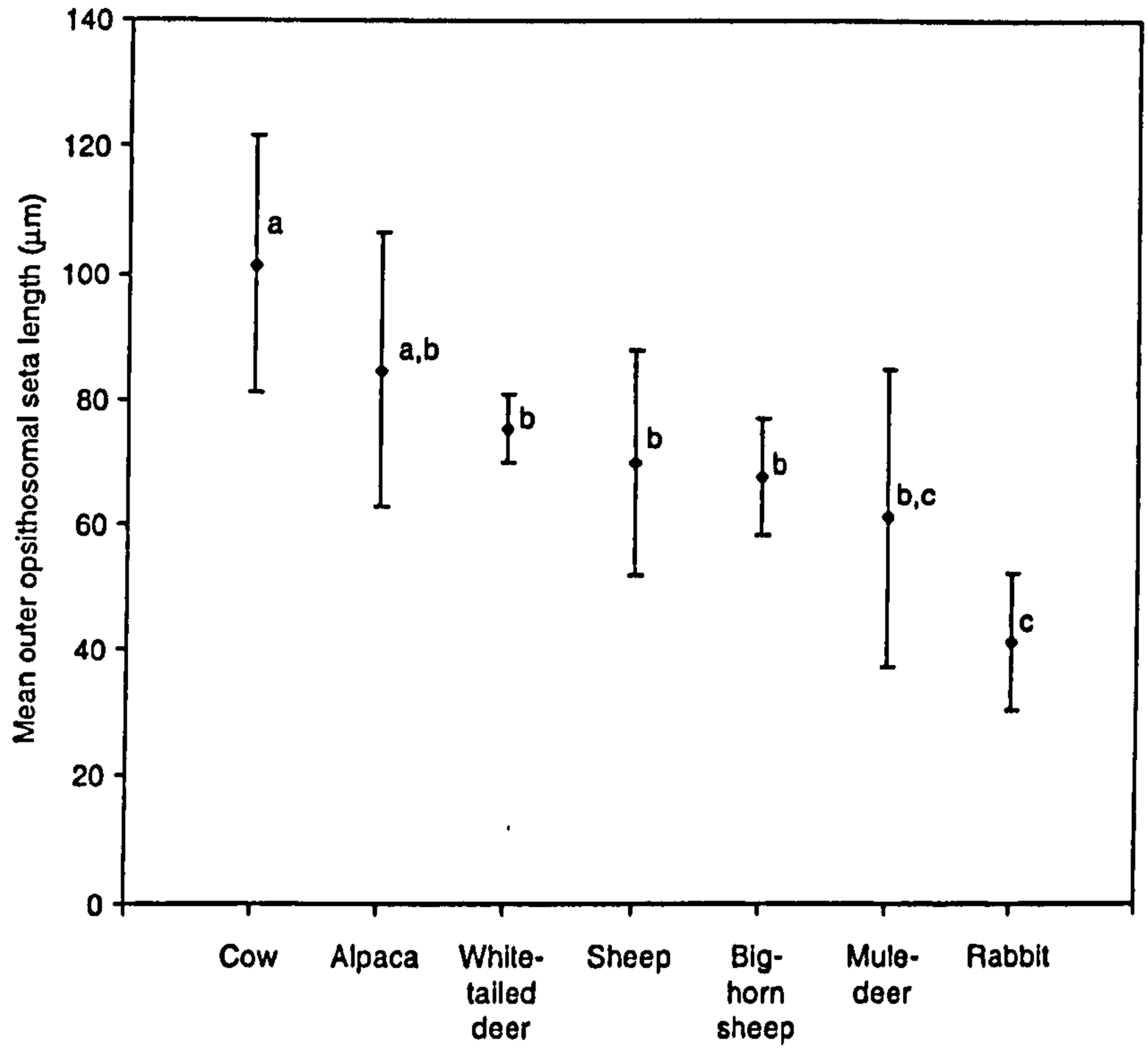

Fig. 5. Mean outer opisthosomal seta length (µm ± SD) of adult female mites collected from a range of host species. Letters indicate points between which there is no statistically significant difference ($F_{6,224} = 50.93$, $P < 0.001$).

mites from rabbit and mule deer and no significant difference between mites from cattle or alpaca. When the outer opisthosomal seta lengths were considered for all individual mite samples, again a large amount of variation between mites from the same host species was evident (Fig. 6). As was the case for the male mites, there was a significant difference in seta length between the cattle isolates from Belgium and the U.S.A. ($t_{45} = -2.502$, $P = 0.016$). There was a significant difference between outer opisthosomal seta length from different sheep isolates ($F_{14,82} = 2.41$,

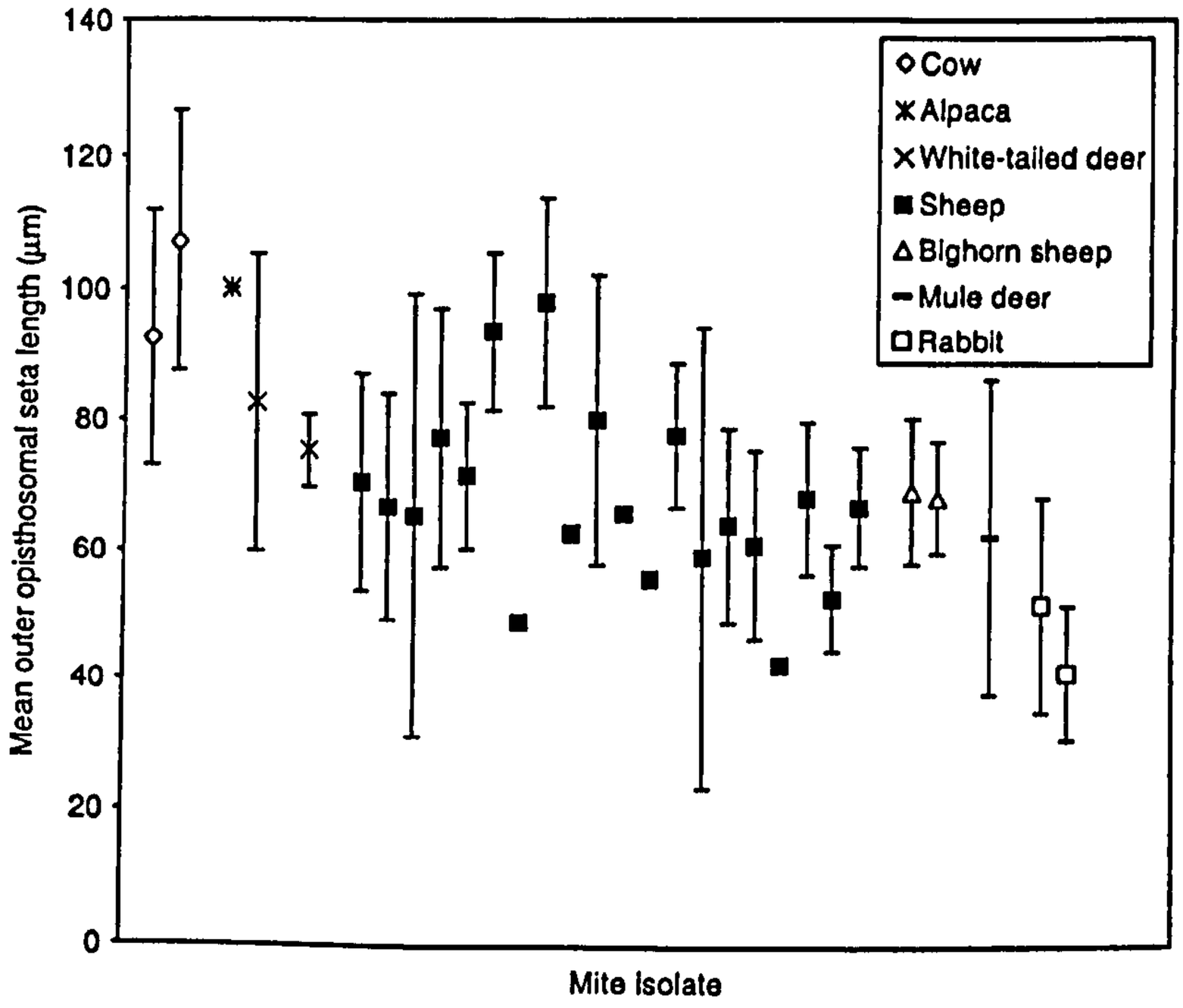

Fig. 6. Mean outer opisthosomal seta length (µm ± SD) of adult female *Psoroptes* mites of all isolates examined.

$P = 0.007$). However, there were no significant differences between isolates from the other host species.

Second internal transcribed spacer region sequencing analysis

Full sequences of the ITS-2 fragment were achieved for 26 of the 41 samples for which amplification was attempted. The remaining 15 samples either failed to amplify with either set of ITS-2 primers, or the amplification was too weak to enable sequencing to be carried out. All 26 *Psoroptes* ITS-2 sequences were aligned with the three published sequences. The ITS sequences amplified were between 325 bp (HUO primers) and 425 bp (RIB primers) long, with a total of 16 polymorphic sites (= 3.75–4.9% polymorphism).

A parsimony-based phylogram was then generated using available mite samples from ITS-2 sequencing data (Fig. 7). Only three pairs of samples clustered separately from the others. These were mites originating from infected sheep in

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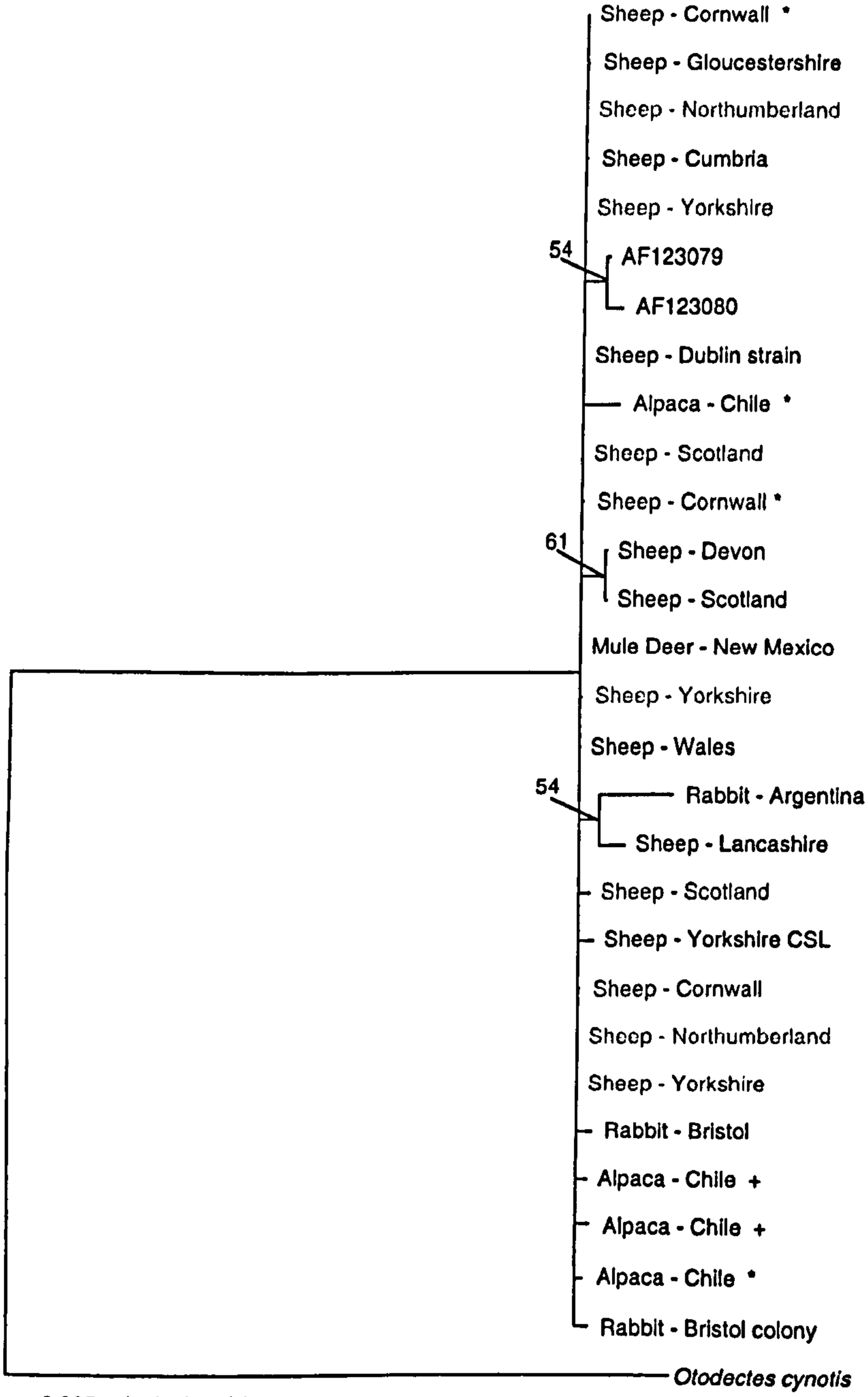

Fig. 7. Phylogram of second internal transcribed spacer (ITS-2) sequences from *Psoroptes* samples, rooted to *Otodectes cynotis*. Bootstrapped consensus tree, bootstrap values > 50% shown on supported branches. Samples from animals within a single herd or wildlife reserve are highlighted with a matching colour. Sequences from the same mite using different primers are marked with matching symbols. AF123079 and AF123080 are published sequences included for sequence identification purposes.

Scotland and Devon, mites from an infected sheep in Lancashire and a rabbit in Argentina, and the two previously published *P. ovis* sequences downloaded from the EMBL database. The sequence alignment is available from JRS (j.r.stevens@ex.ac.uk).

Microsatellite analysis

Amplification was achieved for all 41 samples for seven of the markers, *Psor02*, *Psor04*, *Psor05*, *Psor07*, *Psor11*, *Psor14* and *Psor16*. The two remaining markers, *Psor01* and *Psor13*, failed to amplify most of the new world samples. The phylogram generated from these data us shown in Fig. 8. The phylogram shows several consistent clusters of samples. Samples from two flocks, one in Northumberland and one in Cornwall, group together, and some of the samples from the San Andres Wildlife Refuge, New Mexico group together. Similarly, the two samples from Chilean alpacas cluster together. Two other groupings also remain consistent after bootstrapping; two sheep samples, from South Uist and the Dublin laboratory strain, and a group of sheep samples from a variety of British flocks.

Discussion

The exoskeleton of astigmatid *Psoroptes* mites is largely unsclerotized and, as a result, one potential problem in studies of morphology is the effect of fixation and mounting media on the mites. The effects of fixation on 13 morphological characters of *Psoroptes* mites was considered by Reese *et al.* (1996). Fixation in alcohol was found to result in significant reductions in body size measurements, size decreasing with increasing time of fixation, but the concentration of alcohol had no effect. Mounting in Hoyer's medium had the opposite effect on body size. Changes in

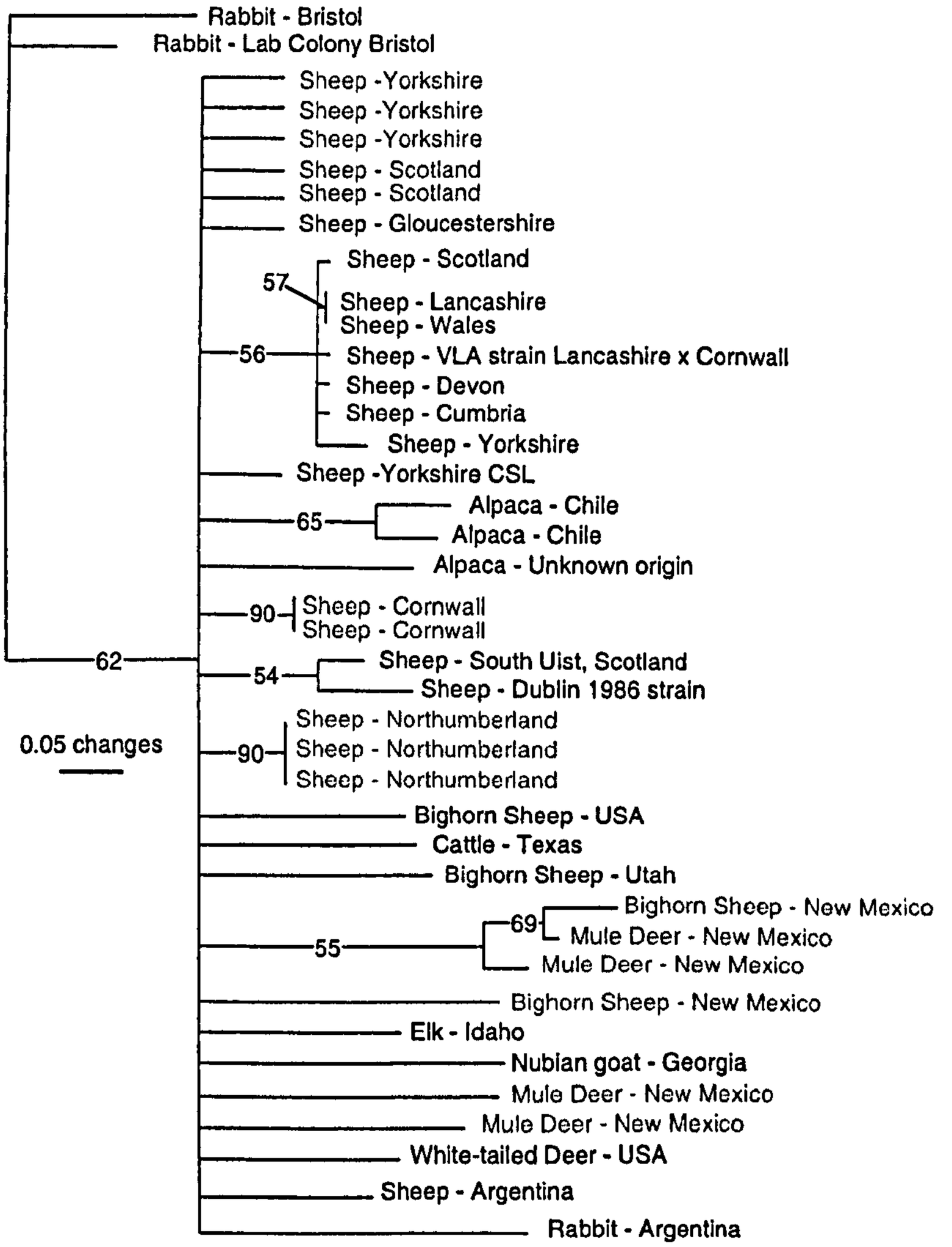

Fig. 8. Phylogram of *Psoroptes ovis* samples created from microsatellite data. Bootstrapped consensus tree, bootstrap values > 50% are shown on supported branches. Samples from animals within a single herd or wildlife reserve are marked with a matching symbol.

measurements as a result of fixation were most apparent in characters that were determined the most by internal tissues, such as body width or length. However, in characters including outer opisthosomal seta length, there was no effect of fixation. Consistent methodology was advocated in morphometric studies by Reese *et al.* (1996) and this was adopted in the present investigation.

The results of this study show that both male and female adult *Psoroptes* mite populations from some different host species can be distinguished by their morphological characters, with outer opisthosomal seta length being the most important character, as suggested by Sweatman (1958). However, when mites are examined from a wide range of host species, considerable variation in character measurements is apparent. In this study, mites from elk and cattle, which would be identified as *P. cervinus* and *P. ovis*, respectively, using Sweatman's key, here appear almost identical in terms of outer opisthosomal seta length. Outer opisthosomal seta lengths of body mites from cattle and sheep were found to be significantly different from each other in both male and female mites, although according to Sweatman's key they should both be described as *P. ovis*. However, significant differences were also found between mites from the same host species, but this was not necessarily a result of differing geographical locations. Differences were seen in male and female mites isolated from cattle from the U.S.A. and Belgium, and also in male mites from two rabbit samples, both from Bristol, U.K. Few morphological differences were found between adult male mites from sheep from all over the U.K. and Ireland but differences were found in the female mites, although this did not appear to correspond to host location.

From these measurements, although there appears to be some broad underlying relationship between morphology and host species, clearly these relationships are weak and for most hosts, interspecific differences are not greater than the morphological variation seen between samples from the same host species, supporting the conclusions of Boyce *et al.* (1990). As a result, it would seem most plausible that the character measurements recorded here are not fixed, but are phenotypically plastic, representing the conditions under which mites have developed and related perhaps to the location on the host, temperature, the age of the infection and possibly the immune status of the host, as suggested by Bates (1999). Host-related phenotypic differences are well known in parasitic mites. For example, the mites *Unionicola poundsi* and *U. lasellai*, which were originally identified as a single species, are found on different freshwater mussel hosts, and adult mites are separated by the shape of particular tarsal claws and setae. Host transfer experiments showed that these morphological characters are dependent on the host species on which the mites moult from the nymphal to adult stages (Downes, 1990). Phenotypically host-adapted mite populations have also been described classically in *Sarcoptes* (Fain, 1994).

The molecular analysis looked initially at the ITS-2 spacer region. This region appears well suited to the phylogenetic study of species groups (McLain *et al.*, 1995; Fukunaga *et al.*, 2000), as it is a non-coding transcribed portion of DNA and hence has been shown to be evolving at a higher rate than the coding regions of the genes on either side (Cruickshank, 2002). Additionally, its location between two highly conserved genes results in a high level of cross-species utility of the primers designed to amplify the region. The ITS-2 sequencing phylogram (Fig. 7) shows very little structure within the Psoroptidae mites; only three pairs of samples group together and of these, bootstrap support is at best only weak (54–61%). These were mites originating from infected sheep in Scotland and Devon, mites from an infected sheep in Lancashire and a rabbit in Argentina, and the two previously published *P. ovis* samples from the EMBL database. The last two samples originate from a sheep host, but information about their geographical location was unavailable. The reasons for the other consistent groupings are unclear. Given that other samples in the collection came from the same host species and similar geographical locations, there is no obvious pattern to the groupings seen in the phylogram. As alluded to previously (Zahler *et al.*, 1998; Ramey *et al.*, 2000), the paralogous nature of this multicopy rRNA gene could possibly account for the lack of meaningful patterns observed within *Psoroptes* ITS-2 data, but, in common with these previous authors, lack of any correlation of sequence-based clades with host or parasite geographical origins do not appear to offer any support for cryptic species within these *Psoroptes* specimens. Overall, this lack of apparently meaningful variation suggests that this kind of sequence data, although excellent for highlighting differences and relationships between genera, is not ideal for investigating the potential differences within genera, or for epidemiological tracking of mite populations.

The microsatellite analysis shows a greater degree of resolution between samples. Several groupings are supported by bootstrap analysis, most grouping individuals from the same host population. Similarly, mites from diverse host animals within a shared geographical range, such as those from the San Andres National Wildlife Refuge, New Mexico, are consistently grouped together. This implies an isolated local population of mites being maintained on the various wildlife species in the Refuge. The extent of this isolation is questioned by the presence of one sample from the Refuge that does not cluster with the others. However, the presence of mites from different host species seems to confirm the cross-infectivity of *P. ovis* as suggested by Ramey *et al.* (2000). In contrast, many of the British mite samples show no pattern of grouping at all, suggesting a countrywide population of genetically indistinguishable mites. The only interesting exception to this is a cluster of two samples, one from South Uist in Scotland and one from the Department of Agriculture, Food and Fishery, Dublin. These samples have been isolated from other sources of sheep scab for 14 and 19 years, respectively. This chronological isolation may mean that the Scottish island sample may be a genetic remnant of the mites that brought sheep scab back to Britain

from Ireland in the mid-1970s, thus explaining their similarity.

Overall, the data presented here suggest that morphological variation, most notably in the length of the outer opisthosomal setae, has only a very approximate relationship with host species, and the variation between mite populations within a host type may be as great as the variation between mites from different host types. The data suggest that Sweatman's (1958) putative five species are not sufficiently different or genetically isolated to support the claim that they should be classified as distinct. Morphological differences appear more likely to be the result of adaptation to the local microenvironment. The one exception, however, may be *P. natalensis*. *Psoroptes natalensis* from South Africa was first described from mites found on cattle and has opisthosomal setae that are quite different to other populations, flattened and blade-like at the distal end (Hirst, 1922; Bates & Sayers, 2002). Samples of *P. natalensis* could not be obtained for the present study.

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References

Babcock, O.G. & Black, W.L. (1933) The common sheep scab mite and its control. *Texas Agricultural Experiment Station Bulletin*, 79, 1–34.

Bates, P. (1999) Inter- and intra-specific variation within the genus *Psoroptes* (Acari: Psoroptidae). *Veterinary Parasitology*, 83, 201–217.

Bates, P. & Sayers, R. (2002) The male L_4 outer opisthosomal seta (L_4OOS) of psoroptic mites as a marker for speciation and virulence. *Cost Action 833: Mange and Myiasis of Livestock* (ed. By M. Good, M. J. Hall, B. Losson, D. O'Brien, K. Pithan and J. Sol). European Communities, Belgium; University of Bari, Italy.

Berriatua, E., French, N.P., Broster, C.E., Morgan, K.L. & Wall, R. (2001) Effect of infestation with *Psoroptes ovis* on the nocturnal rubbing and lying behaviour of housed sheep. *Applied Animal Behaviour Science*, 71, 43–55.

Bisdorff, B., Milnes, A. & Wall, R. (2005) Prevalence and regional distribution of scab, lice and blowfly strike in sheep in Great Britain. *Veterinary Record*, xx, xx–xx (in press).

Blake, B.H., Bay, D.E., Meola, S.M. & Price, M.A. (1978) Morphology of the mouthparts of the sheep scab mite, *Psoroptes ovis*. *Annals of the Entomological Society of America*, 71, 289–294.

Boyce, W.M. & Brown, R.N. (1991) Antigenic characterization of *Psoroptes spp.* (Acari: Psoroptidae) mites from different hosts. *Journal of Parasitology*, 77, 675–679.

Boyce, W., Elliott, L., Clark, R. & Jessup, D. (1990) Morphometric analysis of *Psoroptes spp.* mites from Bighorn sheep, Mule deer, cattle and rabbits. *Journal of Parasitology*, 76, 823–828.

van den Broek, A.H. & Huntley, J.F. (2003) Sheep scab: the disease, pathogenesis and control. *Journal of Comparative Pathology*, 128, 79–91.

van den Broek, A.H.M., Huntley, J.F. & Halliwell, R.E.W. (2003) Cutaneous hypersensitivity reactions to *Psoroptes ovis* and Der p 1 in sheep previously infested with *P. ovis* – the sheep scab mite. *Veterinary Immunology and Immunopathology*, 91, 105–117.

Corke, M.J. & Broom, D.M. (1999) The behaviour of sheep with sheep scab, *Psoroptes ovis* infestation. *Veterinary Parasitology*, 83, 291–300.

Cruickshank, R.H. (2002) Molecular markers for the phylogenetics of mites and ticks. *Systematic and Applied Acarology*, 7, 3–14.

Downes, B.J. (1990) Host-induced morphology in mites: implications for host-parasite coevolution. *Systematic Zoology*, 39, 162–168.

Essig, A., Rinder, H., Gothe, R. & Zahler, M. (1999) Genetic differentiation of mites of the genus *Chorioptes* (Acari: Psoroptidae). *Experimental and Applied Acarology*, 23, 309–318.

Evans, L.M., Dawson, D.A., Wall, R., Burke, T. & Stevens, J.R. (2003) Isolation of *Psoroptes* scab mite microsatellite markers (Acari: Psoroptidae). *Molecular Ecology Notes*, 3, 420–424.

Fain, A. (1994) Adaptation, specificity and host parasite coevolution in mites (Acari). *International Journal for Parasitology*, 24, 1273–1283.

Hering, E. (1838) Die Kräzmilben der thiere und einige verwandte arten, nach eigenen untersuchungen beschrieben. *Nova Acta Physico-Medica Acadamiae Caesareae Leopoldino-Carolinae Nature Curiosorum*. Tomi 18, Pars 2, Vratislaviae und Bonn.

Hirst, S. (1922) Mites injurious to domestic animals. *British Museum (Natural History) Economic Series*, No. 13. British Museum, London.

Lange, R.E., Sandoval, A.V. & Meleney, W.P. (1980) Psoroptic scabies in bighorn sheep (*Ovis canadensis mexicana*) in New Mexico. *Journal of Wildlife Diseases*, 16, 77–82.

Lohse, J., Rinder, H., Gothe, R. & Zahler, M. (2002) Validity of species status of the parasitic mite Otodectes cynotis. *Medical and Veterinary Entomology*, 16, 133–138.

McLain, D.K., Wesson, D.M., Oliver, J.R., J.H. & Collins, F.H. (1995) Variation in ribosomal DNA internal transcribed spacers 1 among eastern population of *Ixodes scapularis* (Acari: Ixodidae). *Journal of Medical Entomology*, 32, 353–360.

Meleney, W.P. (1967) Experimentally induced bovine psoroptic acariasis in a rabbit. *American Journal of Veterinary Research*, 28, 892–894.

Ochs, H., Mathis, A. & Deplazes, P. (1999) Single nucleotide variation in rDNA ITS-2 differentiates *Psoroptes* isolates from sheep and rabbits from the same geographical area. *Parasitology*, 119, 419–424.

Ramey, R.R., Kelley, S.T., Boyce, W.M. & Farrell, B.D. (2000) Phlogeny and host specificity of psoroptic mange mites

(Acarina: Psoroptidae) as indicated by ITS sequence data. *Journal of Medical Entomology*, 37, 791–796.

Reese, N.E., Boyce, W.M., Gardner, I.A. & Nelson, D.M. (1996) Fixation affects morphometric characters of *Psoroptes cuniculi* mites (Acari: Psoroptidae). *Journal of Medical Entomology*, 33, 835–838.

Roberts, I.H., Blachut, K. & Meleney, W.P. (1971) Oversummering location of scab mites, *Psoroptes ovis*, on sheep in New Mexico. *Annals of the Entomological Society of America*, 64, 105–108.

Sanders, A., Froggatt, P., Wall, R. & Smith, K.E. (2000) Life-cycle stage morphology of *Psoroptes* mange mites. *Medical and Veterinary Entomology*, 14, 131–141.

Shilston, A.W. (1915) Sheep scab. Observations on the life-history of *Psoroptes communis var ovis*, and some points connected with the epizootiology of the disease in South Africa. *The Third and Fourth Reports of the Director of Veterinary Research*, pp. 70–98. Department of Agriculture, Union of South Africa, Pretoria.

Siegfried, E., Ochs, H. & Deplazes, P. (2004) Clinical development and serological antibody responses in sheep and rabbits experimentally infested with *Psoroptes ovis* and *Psoroptes cuniculi*. *Veterinary Parasitology*, 124, 109–124.

Sinclair, A.N. & Kirkwood, A.C. (1983) Feeding behaviour of *Psoroptes ovis*. *Veterinary Record*, 112, 65.

Stockman, S. & Berry, A.H. (1913) The *Psoroptes communis ovis*: Some observations on ova and ovipositing. *Journal of Comparative Pathology and Therapeutics*, 26, 45–50.

Strong, K.L. & Halliday, R.B. (1992) Biology and host specificity of the genus *Psoroptes* Gervais (Acarina: Psoroptidae), with reference to its occurrence in Australia. *Experimental and Applied Acarology*, 15, 153–169.

Sweatman, G.K. (1958) On the life history and validity of the species in *Psoroptes*, a genus of mange mites. *Canadian Journal of Zoology*, 36, 905–929.

Tarry, D.W. (1974) Sheep scab: its diagnosis and biology. *Veterinary Record*, 95, 530–532.

Wright, F.C. (1982) Rearing of *Psoroptes cuniculi* Delafond on cattle. *Southwestern Entomologist*, 7, 235–239.

Wright, F.C., Riner, J.C. & Guillot, F.S. (1983) Cross-mating studies with *Psoroptes ovis* (Hering) and *Psoroptes cuniculi* Delafond (Acarina: Psoroptidae). *Journal of Parasitology*, 69, 696–700.

Wright, F.C., Riner, J.C. & Fisher, W.F. (1984) Comparison of lengths of outer opisthosomal setae of male psoroptic mites collected from various hosts. *Journal of Parasitology*, 70, 141–143.

Zahler, M., Gothe, R. & Rinder, H. (1995) Genetic evidence against a morphologically suggestive conspecificity of *Dermocentor reticulans* and *D. marginatus* (Acari: Ixodidae). *International Journal for Parasitology*, 25, 1413–1419.

Zahler, M., Essig, A., Gothe, R. & Rinder, H. (1998) Genetic evidence suggests that *Psoroptes* isolates of different phenotypes, hosts and geographic origins are conspecific. *International Journal for Parasitology*, 28, 1713–1719.

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